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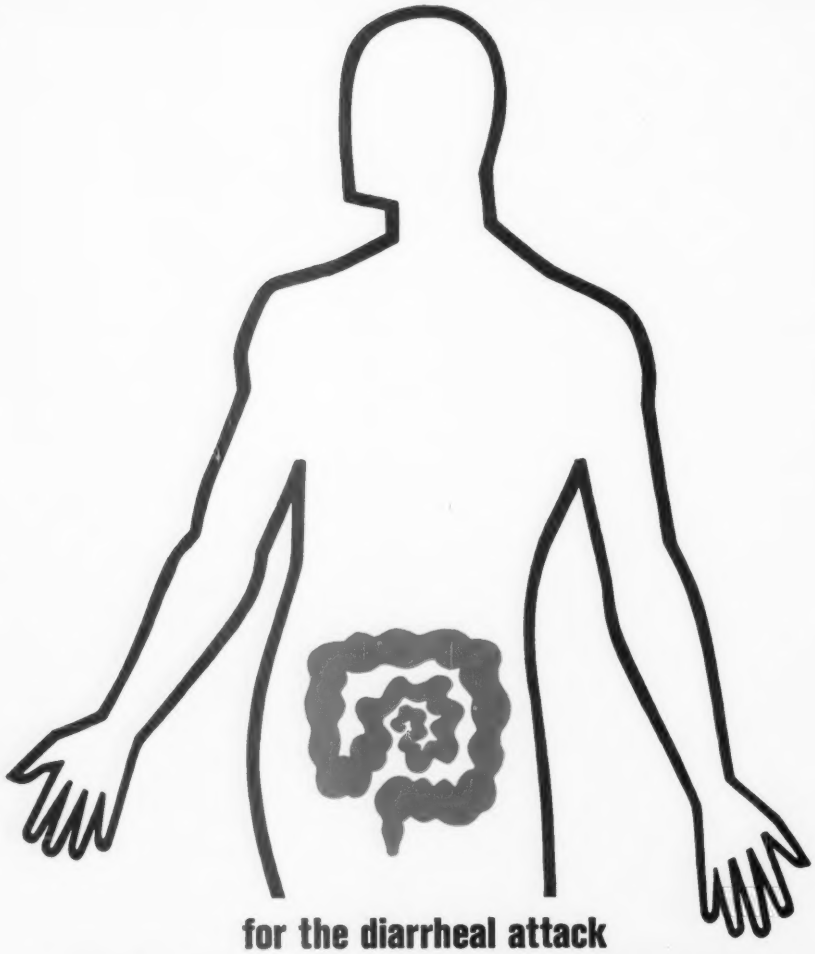

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# STUDIES ON THE RENAL CLEARANCE OF DIFFUSIBLE CALCIUM AND THE ROLE OF THE PARATHYROID GLANDS IN ITS REGULATION§

Calcium is excreted by the kidneys after tubular reabsorption of its diffusible (non-protein-bound) component. There is no evidence to date to suggest that tubular secretion of calcium occurs in the mammalian nephron. In fact, recent studies utilizing the stop-flow technique indicate that calcium is reabsorbed in both the proximal and distal portion of the nephron.[1] The stop-flow studies[1] together with observations on the effect of osmotic diuretics (mannitol, sucrose, NaCl, and $NaHCO_3$) and metabolic inhibitors (phloridzin, dinitrophenol, sodium azide) on calcium excretion, indicate that this ion is reabsorbed by a process of active tubular transport.[2,3,4] The complexing of calcium ion in the extracellular fluids (and therefore in the glomerular filtrate) by organic or inorganic anions such as sulfate,[4] phosphate,[2,3] citrate,[5] or versenate,[2,3] markedly decreases the tubular reabsorption of calcium and may increase calcium excretion from a normal value of 1-5 per cent to 60-80 per cent of the filtered load.[2,3,4] These observations

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§ With the technical assistance of Carol Brewer, Audrey Bishop, and Rose Twiss.

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Presented in part at the annual meeting of the American Society for Clinical Investigation, Atlantic City, May 1958, and the Symposium on Advances in Parathyroid Research, Rice Institute, Houston, Texas, February 1960. This article will also appear as a chapter in the book *The Parathyroids*, R. O. Greed and R. V. Talmage, Eds. C. C Thomas, 1961.

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suggest that calcium is actively reabsorbed in the ionic form, that complexed calcium is largely excreted, and that factors directly altering the tubular reabsorption of calcium do so by affecting the ionic component.

As 95-99+ per cent of the filtered calcium is normally reabsorbed, a change in reabsorption of only 5 per cent could cause a six-fold alteration in calcium excretion. Furthermore, a 5 per cent increase in the filtered load of calcium caused by an increased glomerular filtration rate or a rise in the proportion of the diffusible fraction without a change in per cent reabsorbed could increase the normal daily excretion of calcium four-to five-fold. These illustrations and the problem of variable alterations in calcium complexes indicate the difficulty of interpreting variations in the renal excretion or clearance of calcium.[5,9] However, available evidence supports the concept that for any given filtered load or degree of complex binding, calcium excretion depends on homeostatic mechanisms which regulate active tubular reabsorption and assist in the usual maintenance of ionic serum calcium within normal limits.

Certain observations suggest that parathyroid secretion may be one of the main homeostatic factors controlling tubular reabsorption of calcium. Low phosphorus diets or diets with a high calcium-to-phosphorus ratio are associated with parathyroid atrophy or hypofunction[7,8,9] and significantly increased calcium excretion.[10,11,12] Prolonged intravenous calcium infusions or the rapid liberation of osseous calcium into the extracellular fluids (bone tumors, hyperthyroidism, immobilization) also produce secondary hypofunction of the parathyroids* and hypercalciuria.

Conversely, high phosphorus or low calcium diets (low calcium-to-phosphorus ratio) and extra-osseous disorders leading to calcium depletion (chronic diarrhea, steatorrheas, and vitamin D deficiency) cause significant decreases in calcium excretion and hyperplasia of the parathyroid glands.[7-9,12-18] The above observations suggest that conditions causing parathyroid overactivity are accompanied by a *decreased* renal clearance of calcium, while those causing hypofunction of the parathyroids are accompanied by an *increased* clearance.

Despite hypocalcemia, the excretion of calcium may be normal or even somewhat elevated in primary hypoparathyroidism, particularly of recent origin (postsurgical). Furthermore, one is struck by the frequency of normal calcium excretion in hyperparathyroidism with hypercalcemia, provided that renal function is intact. In fact, to the authors' knowledge,

* In unpublished observations the authors have observed that patients with nonparathyroid disorders causing hypercalciuria with or without hypercalcemia (bone tumors, hyperthyroidism, etc.) may respond to a calcium loading test[18] with a phosphorus excretion pattern resembling hypoparathyroidism.

primary hyperparathyroidism is the only clinical disorder in which hypercalcemia may occur without increased urinary calcium excretion. It was first postulated in 1952 by Talbot that parathyroid hormone increases the tubular reabsorption of calcium.[14] More recently, the studies of Talmage, Kraintz, and Buchanan[15,16] have conclusively shown that parathyroidectomy *increases* and administration of parathyroid extract *decreases* the renal clearance of calcium in the rat. The present study concerns the effects of parathyroid activity on the renal clearance of diffusible calcium in man and dog.

## METHODS AND PROCEDURES

### CLINICAL STUDIES

1. *General methodology.* The studies were performed on 24 healthy males between the ages of 24 and 45 years, 3 hypoparathyroid patients (postsurgical), and 5 patients with hyperparathyroidism secondary to parathyroid adenomas. In all experiments in which infusions of calcium were given, 1 gm. of calcium ion, as the chloride or gluconate salt,* was administered over a four-hour period (8:30 a.m. to 12:30 p.m.). Approximately one-half-hour clearance periods were obtained one hour before, during, and two to four hours after discontinuing the calcium infusion. A mild water diuresis (3-5 cc./min.) was continually maintained, and the subjects reclined throughout, standing only to void.

Venous samples of blood for calcium determination were collected anaerobically (under oil) at the mid-point of each clearance period and transferred to Lavietes ultrafiltration units.[17] Anaerobic ultrafiltration was carried out at room temperature for 14-16 hours. Analysis of the ultrafiltrates for T.C.A. precipitable material was uniformly negative. Samples obtained at two-to four-hour intervals during ultrafiltration showed no significant differences in calcium concentration. The pH of the final ultrafiltrate has always been within 0.1 pH unit of the original anaerobically collected plasma. Replicate determinations of diffusible calcium on the same plasma sample set up in separate ultrafiltration units agreed within $\pm 2$ per cent; however, repeated determinations of diffusible calcium on fasting samples from the same individual, under standardized dietary conditions, for four consecutive mornings disclosed a maximum percentage deviation from the mean of 7.5 per cent.

No significant difference between simultaneously drawn arterial and venous samples was detected. Samples of plasma obtained at one-minute intervals for five minutes after

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* Calcium chloride was administered in the paired studies with and without parathyroid extract. In all other experiments, calcium gluconate was used. Howard, Wilde, and Malvin[1] have recently suggested that the organic anion, gluconate, may interfere with the tubular reabsorption of calcium; and Chen and Neuman[5] observed that calcium administered as the gluconate was excreted more rapidly than calcium as the chloride. It is possible, therefore, that had calcium chloride been used throughout the present studies, the rate of change and the peak calcium clearances attained might have been somewhat lower. However, we are unable to compare the calcium chloride with the calcium gluconate experiments because they were carried out on different patients undergoing different types of studies. For comparison, paired experiments using the chloride and gluconate salts must be done on the same individual.

applying the tourniquet to the arm indicated that *this* duration of venous congestion did not significantly alter the concentration of calcium in the plasma or ultrafiltrate. All syringes and glassware used were washed with double glass-distilled water; all chemical analyses were done in duplicate. Plasma calcium was corrected to its concentration in plasma water by measuring serum proteins by the copper sulfate-specific gravity method. Calcium was analyzed in all fluids by the method of Yanagisawa[18] as modified by Kingsley and Robnett.[19] Phosphorus was determined by the method of Fiske and Subbarow. The technique for inulin clearance measurement and the analysis of inulin and creatinine are described in previous publications from this laboratory.[20]

Crude defatted beef parathyroid glands and human parathyroid glands obtained from autopsy material* were purified through the stage of ultrafiltration by a modification of the method of Rasmussen.[21] The defatted glands were extracted with sub-boiling hydrochloric acid (0.2 per cent) for five minutes (stage I). This acid extract was rapidly filtered, cooled, and re-extracted with cold acetone. The acetone precipitate was discarded and the supernatant (stage II) was ultrafiltered at 4° through a visking membrane for 24 hours. The ultrafiltrate was stage III.

2. *Experiments:* (a). The effect of Lilly parathyroid extract (PTE)** on the clearance of diffusible calcium during a calcium infusion was determined in paired studies (with and without PTE) on seven normal subjects and two patients with hypoparathyroidism maintained on constant calcium and phosphorus diets. Two-hundred units of the Lilly PTE were administered intramuscularly every two hours, beginning two hours before initiating the experiment. A total of 800--1,000 units was usually given.

(b). Six normal subjects had comparable paired clearance studies during calcium infusions after they had ingested either a high calcium-low phosphorus diet (Ca 3 gm., P 0.35 gm.) or a low calcium-high phosphorus diet (Ca 0.2 gm., P 2-3 gm.) for five days. PTE was not administered to this group. All paired studies were separated by an interval of one week. One hypoparathyroid patient on a high calcium-normal phosphorus diet received only one calcium infusion without a paired study.

(c). The acute effect (three-five hours) of a single intramuscular injection of the beef and human extract on calcium clearance was observed in the three hypoparathyroid patients.

(d). Five normal subjects were maintained on a constant calcium and phosphorus diet (Ca 1 gm., P 1.5 gm.) for six-seven days. After two control days, 200 units of Lilly PTE were given intramuscularly every six hours for two or three days; two recovery days followed. Urine was collected in 12- or 24-hour periods, and bloods were collected at the beginning, mid-point, and end of each period for the determination of calcium clearance.

(e). Four normal subjects on constant calcium and phosphorus intakes (Ca 1 gm., P 1.5 gm.), after two control days received 20-30 gm. of calcium carbonate orally for

* The authors wish to thank Dr. Dorothy Tatter, Pathologist, Los Angeles County Hospital, for her invaluable assistance in obtaining the parathyroid glands from autopsy material.

** The authors gratefully thank Dr. Raymond M. Rice of the Lilly Research Laboratories for the generous supplies of Lilly Parathyroid Extract.

three days, followed by four recovery days. Calcium carbonate ingestion was then resumed for an additional three days accompanied by 800 units of Lilly PTE per day. Appropriate blood and urine collections were obtained to determine the relationship of the calcium concentration in the serum to its urinary excretion.

(f). Five patients with primary hyperparathyroidism were studied before and after adenoma removal for alterations in calcium clearance.

TABLE 1. THE EFFECT OF DIET AND PARATHYROID EXTRACT ON THE ENDOGENOUS CLEARANCE OF DIFFUSIBLE CALCIUM*

| *Patient* | $\frac{C_{CA}}{C_{I_n}}$ X 100† | *Serum calcium (mg.%)* | $\frac{C_{CA}}{C_{I_n}}$ X 100 | *Serum calcium (mg.%)* |
|---|---|---|---|---|
| | *High calcium-Low phosphorus* | | *Low calcium-High phosphorus* | |
| Av. | 2.0 | 9.0 | 0.3 | 9.7 |
| Bu. | 3.6 | 8.9 | 1.8 | 8.4 |
| Ro. | 5.2 | 9.0 | 1.0 | 9.0 |
| Ke. | 5.0 | 9.2 | 1.0 | 8.2 |
| Ga. | 3.3 | 10.0 | 0.5 | 8.7 |
| Mo. | 2.8 | 10.0 | 0.2 | 9.0 |
| | *Without PTE* | | *With PTE* | |
| Ho. | 2.5 | 9.4 | 1.6 | 9.0 |
| Co. | 5.8 | 9.2 | 2.8 | 9.8 |
| Ma. | 0.7 | 8.2 | 0.2 | 8.6 |
| Fo. | 2.8 | 9.1 | 1.5 | 9.1 |
| Sh. | 4.7 | 9.8 | 2.0 | 10.0 |
| Na. | 1.8 | 9.3 | 1.1 | 11.8‡ |
| Me. | 8.0 | 9.2 | 1.8 | 9.0 |
| *Hypoparathyroid Patients* | | | | |
| Bu. | 21. | 6.5 | 0.8 | 6.8 |
| Ma. | 1.6 | 7.1 | 0.7 | 8.5 |

* The mean of two control periods prior to the infusion of calcium.

† $\frac{\text{Calcium clearance}}{\text{Inulin clearance}}$ X 100.

‡ This subject received PTE for two days prior to as well as on the experimental day.

**ANIMAL EXPERIMENTS**

Six parathyroidectomized and two sham-operated dogs were studied.

(a). The immediate effect of parathyroidectomy on serum calcium levels and calcium clearances were followed in six animals.

(b). In four parathyroidectomized dogs, the acute effects of intramuscular injection of beef and human parathyroid extract on calcium clearance were studied.

In all experiments the "clearance" of calcium was calculated as the ratio of the observed clearance to that of the simultaneously measured inulin or creatinine $\left(\frac{C_{Ca} \times 100}{C_{In}}\right)$. This represents the per cent of the filtered load excreted or the calcium cleared per 100 cc. of glomerular filtrate in cc. per minute. This calculation standardized

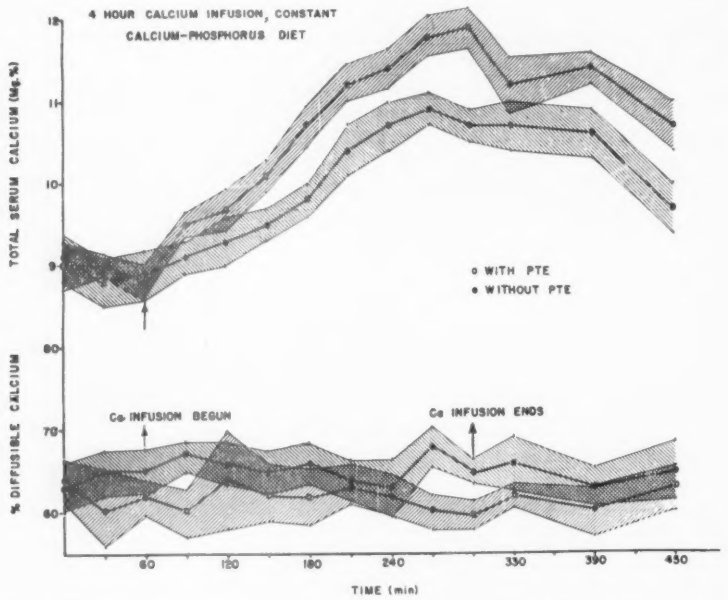


FIG. 1. The effect of a constant infusion of calcium on mean total and diffusible (%) calcium of the serum. The shaded areas represent standard errors of the mean values.

the clearances for variations in size of the individuals and corrected for changes in filtered load not due to changes in plasma concentration of diffusible calcium.

The effect of all the studies mentioned above on the metabolism and excretion of phosphorus will be published separately.[22]

## RESULTS

### THE EFFECT OF DIET AND LILLY PTE ON THE ENDOGENOUS CLEARANCE OF DIFFUSIBLE CALCIUM

Table 1. Endogenous calcium clearances were obtained during the control periods prior to the infusion of calcium. The mean clearance on the high calcium-low phosphorus diets was 3.7, while on the low calcium-high phosphorus diets it was 0.8 (Group b, page 5). The mean clearance on the

1 Gm. calcium-1.5 Gm. phosphorus diet without PTE was 3.8; with PTE it was 1.6 (Group a, page 5). The differences in clearances caused by diet and PTE are significant at the 1 and 5 per cent levels, respectively, without a statistically significant difference in serum values.

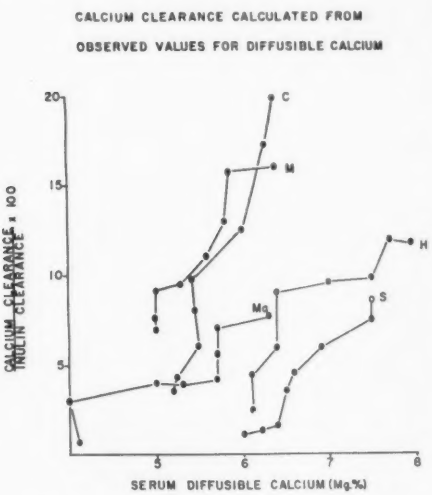


FIG. 2. The renal clearance of calcium calculated from the observed values of diffusible calcium in the ultrafiltrate. Each curve represents a separate subject.

Following his paired study, it was determined that patient Me. (Table 1) probably had idiopathic hypercalciuria. This accounted for his high initial clearance value. The number of subjects studied does not allow us to calculate a reliable mean endogenous calcium clearance for a heterogenous population. This is evident from the overlap of the clearances of the subjects on the high calcium and normal calcium diets. The clearances of two hypoparathyroid patients were within the range observed in the control subjects on normal calcium and phosphorus diets despite distinctly reduced levels of serum calcium. These patients had not been receiving vitamin D.

**THE EFFECT OF CALCIUM INFUSIONS ON THE RENAL CLEARANCE AND SERUM PARTITION OF CALCIUM**

Figure 1 illustrates the change in total and per cent diffusible calcium in the serum before, during, and after the infusion (Group a, page ). The subjects on a normal calcium and phosphorus diet showed an approximate increment in the total serum calcium of 2 mg. per cent during the infusion with a gradual fall toward normal in the subsequent 2½ hours. The mean value for the per cent diffusible calcium (64.0, S.E.±2.5 per cent did not change significantly during this period. This value (64.0) was extremely close to the mean of all subjects observed in the present study (65.1 per cent). The increment in serum calcium when PTE was simultaneously administered was approximately 3 mg. per cent. Again no significant change in per cent diffusible calcium occurred. Although not shown, the pattern

of change in serum calcium was not significantly altered when the subject switched from the high calcium-low phosphorus to the low calcium-high phosphorus diets.

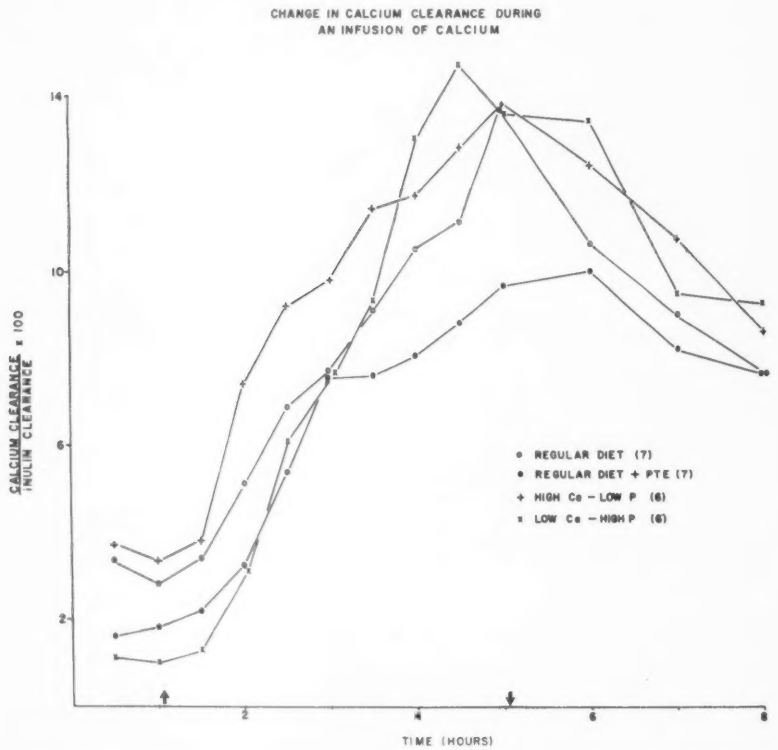


FIG. 3. The diffusible calcium clearance before, during, and after the infusion of calcium plotted against time. Each curve represents the mean of the values obtained from each individual in his respective experimental group. The numbers in parentheses after the group identifications represent the number of subjects. The arrows indicate the beginning and end of the infusion.

The period-to-period variation in per cent diffusible calcium, shown for group a in Figure 1, was great enough in each subject to account for the irregularity in the clearance curves illustrated in Figure 2. These curves were typical of all subjects studied. This degree of variability in per cent diffusible calcium seemed greater than could be accounted for by errors in obtaining the samples, the ultrafiltration, or the chemical analysis. How-

ever, this random variation in diffusible calcium probably represents an undisclosed technical variable rather than a true physiological change in calcium-binding to plasma proteins. Therefore, in each individual experiment, calcium clearance has been calculated using a mean figure for the per cent diffusible calcium. The latter was obtained for *each experiment* by dividing the sum of the determined per cent diffusible calcium from each mid-point blood by the number of samples collected. The use of mean values

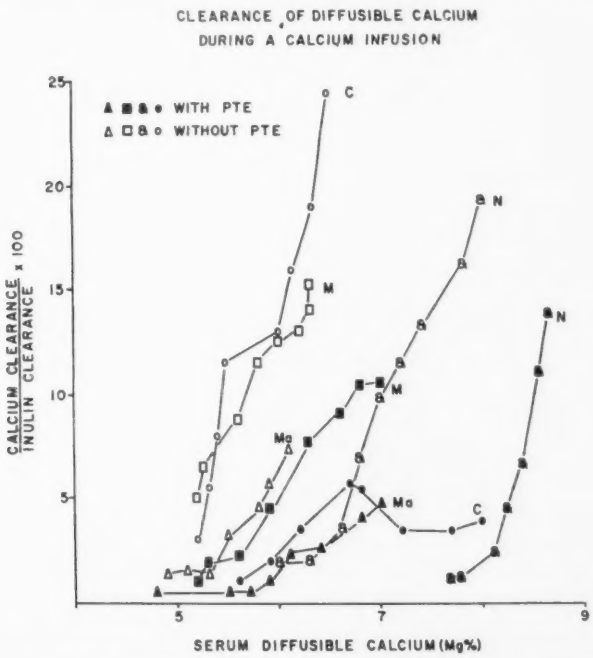


FIG. 4. The effect of parathyroid extract (Lilly) on the diffusible calcium clearance during a calcium infusion. The curves represent the paired experiments on four subjects.

for per cent diffusible calcium did not alter the nature of the results obtained.

In all subjects, the four-hour infusion of calcium caused a progressive rise in calcium clearance. Mean values for the normal subjects at the end of the infusion ranged from 10 to 15 units. The clearances began to decrease soon after terminating the infusion (Fig. 3). This pattern could be correlated with the simultaneous changes in serum calcium or filtered load of this ion (Fig. 1). The decrease in the clearance of calcium that accompanied

TABLE 2. COMPARISON OF CALCIUM CLEARANCE AT COMPARABLE CONCENTRATIONS OF DIFFUSIBLE CALCIUM DURING THE INCREASE AND DECREASE IN SERUM CALCIUM

| *Patient* | *Serum diffusible calcium (mg.%)* | *CCa* ratio with increasing serum calcium* | *CCa* ratio with decreasing serum calcium* |
|---|---|---|---|
| Ho. | 6.3 | 9.2 | 8.5 |
| | 6.7 | 9.4 | 9.1 |
| | 7.1 | 14.9 | 11.2 |
| Ma. | 4.9 | 3.0 | 6.3 |
| | 7.2 | 7.2 | 4.0 |
| Fo. | 6.5 | 4.8 | 4.3 |
| | 6.9 | 7.1 | 7.1 |
| | 7.3 | 15.6 | 8.5 |
| | 7.4 | 15.0 | 12.4 |
| Sm. | 7.0 | 9.0 | 6.6 |
| | 7.6 | 7.9 | 6.4 |
| Co. | 5.6 | 11.3 | 11.9 |
| | 6.1 | 12.7 | 12.2 |
| | 6.3 | 18.3 | 14.0 |
| Me. | 5.5 | 13.5 | 11.2 |
| | 5.8 | 15.7 | 13.1 |
| Av. | 6.3 | 5.7 | 11.3 |
| | 6.4 | 9.8 | 13.5 |
| Bu. | 6.6 | 9.0 | 9.0 |
| | 7.6 | 12.0 | 12.1 |
| Ro. | 7.2 | 12.3 | 11.8 |
| | 7.3 | 9.9 | 17.2 |
| Ke. | 5.8 | 12.8 | 14.8 |
| | 7.0 | 17.0 | 13.6 |
| | 7.8 | 19.8 | 17.2 |
| Ga. | 10.5 | 11.0 | 13.1 |
| | 11.3 | 13.3 | 12.9 |
| Mo. | 7.8 | 4.5 | 4.7 |
| | 8.1 | 5.3 | 5.7 |
| | 8.6 | 6.4 | 6.1 |
| | 8.8 | 6.4 | 6.3 |

* $\frac{\text{Calcium clearance}}{\text{Inulin clearance}} \times 100.$

the falling filtered load differs from the pattern observed following a single large intravenous injection of calcium. In the latter circumstance in both humans [20] and dogs,[2] the maximal clearance is observed one-two hours *after* the peak blood level is attained. This difference may be due to an artifact created by urinary dead space and the extremely rapid changes in serum calcium that accompany a single injection; or it represents a physiological "lag" in the rise in calcium clearance masked by the continuous infusion of calcium used in the present studies. The absence of even a relative increase in calcium clearance after discontinuing the infusion is evident from the data presented in Table 2. For any given concentration of diffusible calcium, the clearance is either the same or somewhat higher on the ascending limb of the clearance curve.

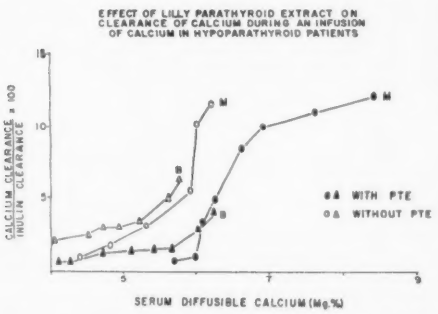


FIG. 5. The effect of parathyroid extract (Lilly) on the diffusible calcium clearance during a calcium infusion in hypoparathyroid patients. The curves represent the paired experiments on two patients.

Variations in the degree of water diuresis during the calcium infusion did not affect the rate of calcium excretion. This was also noted by Chen and Neuman.[2]

1. *The effect of PTE on calcium clearance.* In all subsequent figures only the calcium clearances during the calcium infusion have been plotted. PTE caused a significant reduction in calcium clearance *for any given level of diffusible calcium* (*or filtered load*) in four of the seven normal subjects in this group (Fig. 4) and in two hypoparathyroid patients (Fig. 5). It is apparent that the general pattern of the curves was similar for each individual but differed markedly from subject to subject. In three of the paired studies in this group PTE did not cause a significant reduction in calcium clearance (Fig. 6).*

One hypoparathyroid patient (La.) not receiving PTE in a paired study has been included. Her endogenous and peak calcium clearance (11 and 30.5) were the highest observed in any subject (Fig. 7). She ingested a

* This was not due to inactive extract, at least with regard to phosphorus excretion, for in these three studies PTE caused a marked increase in the clearance of phosphorus.[22]

TABLE 3. TUBULAR REABSORPTION OF CALCIUM (MG./MIN) AS FILTERED LOAD INCREASED

| *Patient* | *Control* | *1 Hour* | *2 Hours* | *3 Hours* | *4 Hours* | *Control* | *1 Hour* | *2 Hours* | *3 Hours* | *4 Hours* |
|---|---|---|---|---|---|---|---|---|---|---|
| | *High Phosphorus—Low calcium diet* | | | | | *High calcium—Low phosphorus diet* | | | | |
| Mo. | 12.9 | 12.7 | 12.8 | 15.4 | 15.9 | 12.8 | 16.0 | 16.4 | 26.3 | 26.6 |
| Av. | 7.4 | 7.5 | 7.2 | 7.4 | 7.8 | 8.1 | 6.1 | 7.5 | 8.8 | 10.5 |
| Bu. | 6.4 | 4.9 | 7.4 | 7.1 | 7.9 | 7.7 | 7.4 | 9.5 | 9.5 | 10.3 |
| Ro. | 4.7 | 5.7 | 6.1 | 5.4 | 6.4 | 5.4 | 4.3 | 5.3 | 4.4 | 7.6 |
| Ga. | 3.6 | 3.9 | 4.7 | 4.9 | 5.1 | 4.5 | 5.9 | 5.2 | 6.2 | 6.4 |
| Ke. | 5.0 | 6.1 | 6.8 | 8.1 | 8.4 | 7.4 | 7.3 | 8.1 | 9.5 | 10.0 |
| | *Standard diet without PTE* | | | | | *Standard diet with PTE* | | | | |
| Ho. | 6.8 | 8.9 | 9.2 | 9.0 | 9.4 | 7.5 | 5.1 | 6.9 | 9.7 | 8.4 |
| Co. | 4.3 | 4.0 | 3.8 | 3.8 | 4.4 | 6.6 | 6.0 | 6.6 | 7.4 | 9.5 |
| Ma. | 7.1 | 6.9 | 7.6 | 7.7 | 8.7 | 6.4 | 7.4 | 8.2 | 8.7 | 9.8 |
| Me. | 6.0 | 6.3 | 6.4 | 7.5 | 7.1 | .. | 6.6 | 7.2 | 7.8 | 8.9 |
| Sh. | 7.4 | 14.0 | 15.8 | 15.5 | 16.6 | 7.0 | 9.9 | 11.7 | 12.8 | 13.2 |
| Fo. | 7.5 | 8.9 | 9.7 | 9.1 | 9.8 | 7.2 | 7.6 | 8.8 | 9.6 | 9.6 |
| Na. | 7.3 | 9.7 | 11.6 | 14.3 | .. | .. | .. | .. | .. | .. |
| | *Hypoparathyroid patients without PTE* | | | | | *Hypoparathyroid patients with PTE* | | | | |
| Bu. | 7.4 | 6.8 | 7.4 | 7.8 | 8.2 | 3.0 | 6.7 | 8.5 | 8.8 | 9.8 |
| Ma. | 4.7 | 5.0 | 5.9 | 6.0 | 7.3 | 7.0 | 7.8 | 8.3 | 9.5 | 9.8 |
| La. | 3.5 | 4.3 | 5.1 | 4.6 | 5.7 | .. | .. | .. | .. | .. |

high calcium (at least 3 Gm.)-normal phosphorus diet with a vitamin D intake not exceeding 10- 15,000 U./day.

2. *The effect of high calcium-low phosphorus and high phosphorus-low calcium diets on calcium clearance.* Earlier studies indicate that these two diets are capable of producing hypofunction and hyperfunction, respectively, of the parathyroid glands.[1,8,9] The marked difference in endogenous calcium

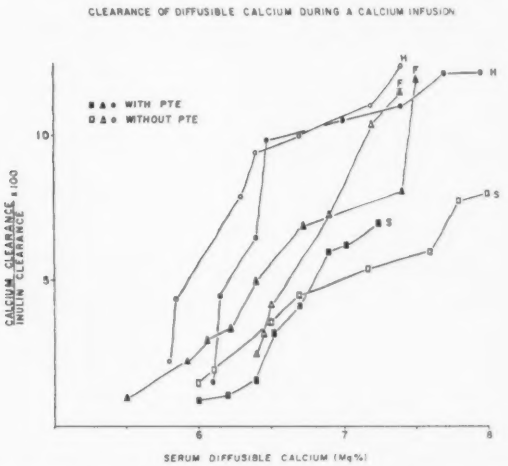


FIG. 6. Three paired experiments during a calcium infusion illustrating the failure of parathyroid extract (Lilly) to depress the clearances.

clearance caused by these two diets had been referred to in an earlier section (Table 1). Raising the serum calcium level by the infusion rapidly eliminated the difference between subjects on a high calcium and low calcium diet (Fig. 8). The difference in the initial slope of the curves on the two diets accounts for the pattern of the mean clearance curves plotted against time in Figure 3.

### THE EFFECT OF BEEF AND HUMAN PARATHYROID EXTRACT ON THE ENDOGENOUS CLEARANCE OF CALCIUM IN HYPOTHYROID PATIENTS AFTER SINGLE OR MULTIPLE INJECTIONS (GROUP C)

Table 4 (1) *Patient Bu.* (24-year-old male). This patient had a total parathyroidectomy for papillary adenocarcinoma. Severe tetany developed two days postoperatively and the clearance of calcium was 2.1, an obviously high figure for a total serum calcium of 6.5 mg.%. A course of AT-10 increased the concentration of calcium in his serum to 8.1 mg.%, and at this

TABLE 4. THE EFFECT OF SINGLE AND MULTIPLE INJECTIONS OF BEEF AND HUMAN PTE ON THE ENDOGENOUS CLEARANCE OF CALCIUM IN HYPOPARATHYROID PATIENTS

| *Patient* | *Period* | | *Duration* | *Total Serum Calcium (mg. %)* | *Diffusible Calcium (mg. %)* | $\frac{C_{Ca} \times 100^*}{C_{In}}$ | $\frac{C_P \times 100^{**}}{C_{In}}$ |
|---|---|---|---|---|---|---|---|
| 1. *Bu.* | | | | | | | |
| Hypoparathyroidism following total thyroidectomy | | | | | | | |
| 3/25/57—Tetany 2 days postoperative | | | 24 hrs. | 6.5 | 4.1† | 2.1‡ | |
| 4/3/57—7 days after starting A.T.-10 | | | 24 hrs. | 8.1 | 5.1† | 3.8‡ | |
| 4/27/57—24 days after A.T.-10 discontinued | | | 24 hrs. | 5.6 | 3.5† | 2.2‡ | |
| 4/29/57—after 2 days of Lilly PTE (800 U./day) | | | 24 hrs. | 8.5 | 5.4† | 0.6‡ | |
| 5/6/57—6 days after PTE discontinued | | | 24 hrs. | 7.1 | 4.5† | 2.5‡ | |
| 2. *Bu.* | | | | | | | |
| Following clearance periods morning of Day 1, | Day 1 | | 30 min. | 7.8 | 4.7 | 4.3 | |
| 200 U. Lilly PTE I.M. every 6 hrs. through | " " | | 30 min. | 7.6 | 4.5 | 3.9 | |
| clearance periods of Day 2. | " " | | 30 min. | 7.5 | 4.5 | 3.5 | 0.5 |
| | Day 2 | | 30 min. | 8.6 | 5.0 | 2.4 | |
| | " " | | 30 min. | 8.6 | 4.8 | 1.9 | |
| | " " | | 30 min. | 8.7 | 5.1 | 2.1 | 7.0 |
| 3. *Ma.* | | | | | | | |
| High Ca-low P diet. No Vit. D. | Day 1 | | 2 hrs. | 7.0 | 4.6 | 2.7 | |
| After clearance period on day 1, 4 injections | Day 2 | | 2 hrs. | 8.4 | 5.7 | 0.9 | |
| Beef PTE, stage 1, I.M. every 6 hrs. | | | | | | | |
| 4. *Ma.* | | | | | | | |
| 2 injections human PTE, stage III, I.M. | | | 30 min. | 6.4 | 4.3 | 0.7 | |
| | | | 30 min. | 6.8 | 4.6 | 0.9 | 1.5 |
| | | PTE→ | 60 min. | 6.2 | 4.2 | 1.1 | |
| | | | 60 min. | 6.5 | 4.4 | 0.4 | |
| | | PTE→ | 60 min. | 7.0 | 4.7 | 0.2 | 36.0 |
| | | | 60 min. | 6.9 | 4.6 | 0.3 | |
| | | | 60 min. | 6.9 | 4.6 | 0.3 | 17.5 |
| 5. *Ma.* | | | | | | | |
| 1 injection human PTE, stage I, I.M. | | | 20 min. | 6.9 | 4.5 | 0.6 | 1.6 |
| | | | 20 min. | 7.2 | 4.7 | 0.6 | |
| | | | 30 min. | | | | 4.2 |
| | | PTE→ | 60 min. | 6.9 | 4.5 | 0.5 | 14.0 |
| | | | 60 min. | 7.0 | 4.6 | 0.3 | 25.6 |
| | | | 60 min. | 6.8 | 4.4 | 0.3 | 16.8 |
| | | | 60 min. | 6.8 | 4.4 | 0.3 | 8.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6. *Ma.* | | | | | | |
| 1 injection beef PTE, stage II, I.M. | | 30 min. | 6.3 | 4.1 | 1.2 | 0.4 |
| | | 30 min. | 6.3 | 4.1 | 0.8 | 0.2 |
| | PTE→ | 30 min. (90§) | 7.0 | 4.6 | 0.3 | 12.8 |
| | | 30 min. (150§) | 7.0 | 4.6 | 0.3 | 32.5 |
| | | 30 min. (300§) | 6.8 | 4.4 | 0.3 | 41.5 |
| 7. *Ma.* | | | | | | |
| 1 injection beef PTE, stage III, I.M. | | 30 min. | 6.5 | 4.2 | 0.6 | 3.7 |
| | | 30 min. | 6.1 | 4.0 | 0.7 | 1.9 |
| | PTE→ | 30 min. (120§) | 6.5 | 4.2 | 0.6 | 35.6 |
| | | 30 min. (180§) | 6.5 | 4.2 | 0.3 | |
| | | 30 min. (210§) | 6.5 | 4.2 | 0.3 | 38.2 |
| | | 30 min. (300§) | 6.4 | 4.2 | 0.6 | 20.4 |
| 8. *La.* | | | | | | |
| High Ca.—normal P diet. No Vit. D. | | 60 min. | 7.1 | 4.6 | 8.4 | |
| 1 injection human PTE, stage I, I.M. | | 60 min. | 6.7 | 4.4 | 8.1 | 6.5 |
| | PTE→ | 60 min. | 6.8 | 4.4 | 8.5 | |
| | | 60 min. | 6.8 | 4.5 | 3.9 | |
| | | 60 min. | 6.8 | 4.4 | 3.2 | 38.0 |
| 9. *La.* | | | | | | |
| 1 injection beef PTE, stage III, I.M. | | 60 min. | 8.4 | 5.5 | 10.2 | 6.8 |
| | | 30 min. | 8.6 | 5.6 | 9.0 | 8.5 |
| | PTE→ | 60 min. | 8.6 | 5.6 | 7.6 | 12.6 |
| | | 60 min. | 9.1 | 5.9 | 2.3 | 34.1 |
| | | 60 min. | 8.6 | 5.6 | 1.2 | 72.5 |
| | | 60 min. | 8.2 | 5.3 | 1.2 | 58.0 |
| | | 60 min. | 8.2 | 5.3 | 1.1 | 49.0 |
| | | 60 min. | 8.1 | 5.3 | 2.0 | 43.0 |

* $\frac{\text{Calcium clearance}}{\text{Inulin clearance}} \times 100.$

** $\frac{\text{Phosphorus clearance}}{\text{Inulin clearance}} \times 100.$

† These diffusible calcium concentrations were calculated from the mean per cent diffusible value of all the ultrafiltrates subsequently determined on this patient.

‡ Calculated from 24-hour calcium and creatinine excretion $\left(\frac{C_{C_a} \times 100}{C_{C_r}}\right)$.

§ Time after injection (min.).

level his calcium clearance was 3.8. Twenty-four days after discontinuing the AT-10 (dihydrotachysterol), the calcium clearance at a serum value of 5.6 mg.% was 2.2. After two days of Lilly PTE (800 U./day, I.M.) the clearance had fallen to 0.6 despite a rise in serum calcium to 8.5 mg.%. Withdrawal of PTE was followed by a rapid fall in serum calcium (7.1 mg.%) and a significant *rise* in calcium clearance.[4,8] The patient was maintained on a high calcium—low phosphorus diet *without vitamin D* for approximately three weeks and the experiment tabulated in Table 4 (2) was done. Again, Lilly PTE caused a significant decrease in calcium clearance despite an increase in serum calcium.

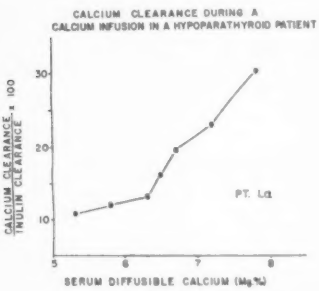


FIG. 7. The effect of a calcium infusion on the diffusible calcium clearance in a hypoparathyroid patient.

Table 4 (3) *Patient Ma.* (45-year-old female). This patient with chronic postsurgical hypoparathyroidism received an I.M. injection every six hours of beef PTE prepared in our laboratory (stage I) after having obtained the two-hour control clearance on the morning of day 1. Comparable samples were collected on the morning of day 2. Serum calcium increased 1.4 mg.%, while calcium clearance decreased from 2.7 to 0.9.

The subsequent acute experiments on patients Ma. and La. (50-year-old female, postsurgical hypoparathyroidism) have been tabulated in Table 4 (4-9) and illustrated in Figures 9 and 10. A single acute intramuscular injection of beef or human PTE caused a moderate-to-marked decrease in calcium clearance in each experiment. The lowest clearance values were obtained in approximately three hours. Phosphorous clearances have been included in Table 4 for comparison. The concentration of calcium in the serum did not rise significantly during these acute experiments. Purification of beef or human extract through the stage of ultrafiltration (stage III)[21] did not cause a loss of either the phosphate-diuretic or calcium-antidiuretic "properties."

## CALCIUM CLEARANCE IN NORMAL SUBJECTS DURING AND AFTER THE PROLONGED ADMINISTRATION OF LILLY PTE

The authors have observed that a 12- to 24-hour period of temporary functional hypoparathyroidism (as measured by changing phosporus clearances) follows the iatrogenic "hyperparathyroidism" produced by multiple

injections of Lilly PTE for two days.* It seemed of interest to note the effect of this procedure on the renal clearance of calcium. This was done on five normal subjects. The results are tabulated in Table 5 and Figure 11. In all five subjects, the clearance of calcium rose significantly in the 12- to 24-hour period of functional hypoparathyroidism following withdrawal of the PTE (stage III). Despite the increase in serum during the injection of the extract, only one subject (J.S.) demonstrated a rise in calcium clearance during period II.

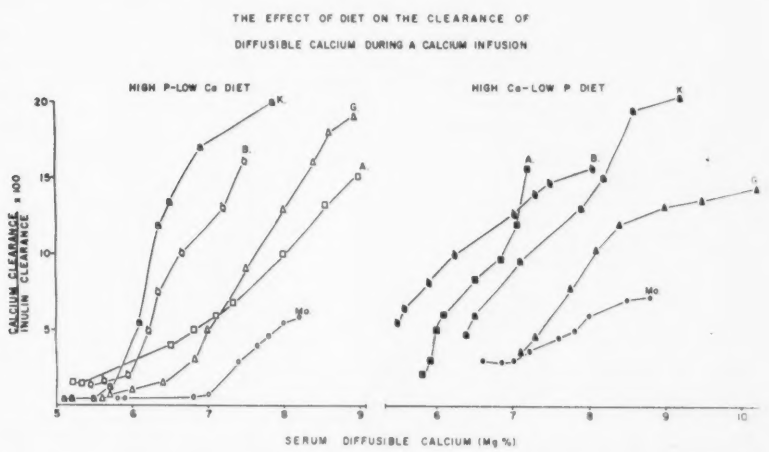
THE EFFECT OF DIET ON THE CLEARANCE OF
DIFFUSIBLE CALCIUM DURING A CALCIUM INFUSION
HIGH P-LOW Ca DIET
HIGH Ca-LOW P DIET
CALCIUM CLEARANCE / INULIN CLEARANCE x 100
SERUM DIFFUSIBLE CALCIUM (Mg %)

FIG. 8. The effect of altered calcium and phosphorus content of the diet on the diffusible calcium clearance during a calcium infusion. The curves represent the paired experiments on five subjects.

## THE EFFECT OF CALCIUM CARBONATE INGESTION WITH AND WITHOUT LILLY PTE ON SERUM CALCIUM AND CALCIUM EXCRETION

These studies were initially done as part of an investigation into the etiology of the milk-alkali (Burnett's) syndrome and have been reported elsewhere in abstract form.[24] The values shown in Figure 12 represent the means of four subjects. The design of the experiment is outlined in the methods and procedure section. Calcium carbonate alone caused a marked increase in urinary calcium and decrease in urinary phosphorus excretion, the latter due primarily to the formation of non-absorbable calcium phos-

*This technique of producing functional hypoparathyroidism was first suggested to the senior author by Dr. Sidney H. Ingbar, Thorndike Memorial Laboratory, Boston City Hospital, Boston, Massachusetts.

TABLE 5. CALCIUM CLEARANCE IN NORMAL SUBJECTS ON CONSTANT NORMAL CALCIUM AND PHOSPHORUS DIETS BEFORE, DURING, AND AFTER THE CHRONIC ADMINISTRATION OF LILLY *PTE*

| *Patient* | *Period* | *Duration* | *Total Serum Calcium (mg. %)* | *Diffusible Calcium (mg. %)* | $\frac{C_{Ca} \times 100}{C_r}$* | $\frac{C_P \times 100}{C_r}$† |
|---|---|---|---|---|---|---|
| 1. J.S. 6/2/58 | Pre-PTE | 24 hrs. | 10.5 | 6.8 | 2.1 | 21.0 |
| | PTE‡ | 12 hrs. | 11.6 | 7.5 | 3.8 | — |
| | | 12 hrs. | 11.6 | 7.2 | 5.8 | 30.3 |
| | Post-PTE | 12 hrs. | 10.2 | 6.6 | 8.7 | 10.0 |
| | | 12 hrs. | 9.7 | 6.3 | 9.7 | 10.7 |
| | | 24 hrs. | 10.0 | 6.5 | 5.1 | — |
| 2. H.K. 1/22/58 | Pre-PTE | 24 hrs. | 9.4 | 6.2 | 3.0 | — |
| | | 24 hrs. | 9.4 | 6.2 | 3.0 | 7.0 |
| | PTE‡ | 24 hrs. | 10.6 | 7.1 | 1.9 | 14.0 |
| | | 24 hrs. | 11.4 | 7.6 | 2.9 | 32.0 |
| | Post-PTE | 12 hrs. | 10.9 | 7.3 | 5.2 | 5.0 |
| | | 12 hrs. | 10.9 | 7.3 | 4.5 | 3.0 |
| | | 24 hrs. | 10.9 | 7.3 | 3.2 | — |
| 3. R.C. 11/10/57 | Pre-PTE | 24 hrs. | 9.2 | 6.2 | 0.6 | — |
| | | 24 hrs. | 9.2 | 6.2 | 1.0 | 18.0 |
| | PTE‡ | 24 hrs. | 9.5 | 7.0 | 0.7 | — |
| | | 24 hrs. | 10.3 | 7.2 | 0.9 | 32.0 |
| | Post-PTE | 12 hrs. | 9.7 | 7.0 | 2.6 | — |
| | | 12 hrs. | 9.8 | 7.0 | 3.1 | — |
| | | 12 hrs. | 9.5 | 6.7 | 4.5 | 7.0 |
| | | 12 hrs. | 9.5 | 6.7 | 2.1 | — |
| 4. P.B. 4/2/58 | Pre-PTE | 24 hrs. | 8.8 | 5.6 | — | — |
| | | 24 hrs. | 9.4 | 5.9 | 2.9 | 12.0 |
| | PTE‡ | 24 hrs. | 10.2 | 6.2 | 2.5 | — |
| | | 24 hrs. | 10.4 | 6.9 | 0.4 | 25.0 |
| | Post-PTE | 12 hrs. | 8.2 | 5.2 | 4.0 | 6.0 |
| | | 12 hrs. | 9.9 | 5.8 | 3.0 | — |
| 5. W.J. 5/3/58 | Pre-PTE | 24 hrs. | 11.2 | 6.7 | 3.0 | — |
| | | 24 hrs. | 11.3 | 7.0 | 3.0 | 11.5 |
| | PTE§ | 24 hrs. | 11.2 | 7.0 | 2.7 | — |
| | | 24 hrs. | 11.9 | 7.8 | 1.7 | — |
| | | 24 hrs. | 12.2 | 8.0 | 2.1 | 22.0 |
| | Post-PTE | 12 hrs. | 11.2 | 7.0 | 6.0 | 5.0 |
| | | 12 hrs. | 10.7 | 6.6 | 3.8 | — |
| | | 12 hrs. | 10.7 | 6.6 | 4.0 | 7.0 |

* $\frac{\text{Calcium clearance}}{\text{Creatinine clearance}} \times 100.$

† $\frac{\text{Phosphorus clearance}}{\text{Creatinine clearance}} \times 100.$

‡ 800 U. Lilly PTE I.M./day for 2 days.

§ 800 U. Lilly PTE I.M./day for 3 days.

TABLE 6. THE EFFECT OF PARATHYROIDECTOMY ON THE RENAL CLEARANCE OF CALCIUM AND PHOSPHORUS IN HYPERPARATHYROID PATIENTS

| *Patient* | *Period* | *Duration* | *Total Serum Calcium (mg. %)* | *Diffusible Calcium (mg. %)* | $\frac{C_{Ca}}{C_{Cr}} X100$* | $\frac{C_{P}}{C_{Cr}} X100$† |
|---|---|---|---|---|---|---|
| Ri. | Preop. | 30 min. | 13.2 | 9.2 | 7.6 | 17.0 |
| | | 30 min. | 13.2 | 9.2 | 7.9 | 16.0 |
| | Immediate postop. | 480 min. | 11.4 | 7.9 | 3.5 | 14.2 |
| | | 480 min. | 10.4 | 7.2 | 1.0 | 0.6 |
| | | 480 min. | 9.2 | — | — | — |
| | | 480 min. | 9.1 | 6.3 | 0.2 | 0.2 |
| Au. | Preop. | 40 min. | 13.4 | 8.7 | 4.0 | 56.0 |
| | | 40 min. | 13.4 | 8.7 | 4.3 | 57.0 |
| Ca. | Preop. | 20 min. | 14.3 | 9.3 | 9.0 | 67.0 |
| | | 20 min. | 14.5 | 9.4 | 9.3 | 68.0 |
| | | 50 min. | 14.4 | 9.4 | 9.5 | 65.0 |
| | Immediate postop. | 370 min. | 13.0 | 8.4 | 7.4 | 65.0 |
| | 4 days postop. | 40 min. | 8.6 | 5.6 | 4.6 | 14.0 |
| | | 40 min. | 8.4 | 5.4 | 4.5 | 22.0 |
| | | 40 min. | 8.8 | 5.6 | 4.3 | 15.0 |
| Ba. | Preop. | 60 min. | 12.0 | 7.8 | 6.9 | 32.0 |
| | | 60 min. | 11.8 | 7.6 | 6.9 | 25.5 |
| | | 60 min. | 11.8 | 7.6 | 6.3 | 23.0 |
| | Postop. 24 hrs. | 12 hrs. | 9.9 | 5.7 | 5.5 | 1.5 |
| | Postop. 40 hrs. | 12 hrs. | 7.9 | 4.7 | 4.4 | 0.1 |
| | Postop. 48 hrs. | 24 hrs. | 9.8 | 6.1 | 3.8 | 0.3 |
| | Postop. 12 days | 24 hrs. | 9.7 | 5.4 | 1.0 | 18.1 |
| | Postop. 2 mo. | 30 min. | 9.0 | 6.0 | 2.4 | 20.0 |
| | Postop. 2 mo. | 30 min. | 9.0 | 5.9 | 2.4 | 20.0 |
| | Postop. 2 mo. (During calcium infusion) | 30 min. | 11.6 | 7.6 | 6.7 | 23.0 |
| | Postop. 6 mo. | 24 hrs. | 9.6 | 5.6 | 0.4 | 24.0 |
| | Postop. 6 mo. | 24 hrs. | 9.6 | 5.6 | 0.5 | 22.0 |
| Bg. | Preop. | 24 hrs. | 11.4 | 7.4 | 4.2 | 26.0 |
| | | 24 hrs. | 11.9 | 7.7 | 3.0 | 26.6 |
| | Postop. 7 hrs. | 11 hrs. | 10.1 | 6.7 | 4.9 | 19.7 |
| | Postop. 12 hrs. | 4 hrs. | 9.9 | 6.4 | 5.6 | 13.8 |
| | Postop. 15 hrs. | 3 hrs. | 9.7 | 6.4 | 5.0 | 11.6 |
| | Postop. 24 hrs. | 6 hrs. | 9.3 | 6.0 | 2.4 | 1.9 |

* $\frac{\text{Calcium clearance}}{\text{Creatinine clearance}} \times 100.$

† $\frac{\text{Phosphorus clearance}}{\text{Creatinine clearance}} \times 100.$

phate in the gastrointestinal tract. Serum calcium increased slightly. When the ingestion of calcium carbonate was combined with PTE administration, the mean serum calcium increased significantly, while calcium excretion was essentially unchanged as compared with calcium carbonate ingestion alone. All four subjects behaved in the same way. The probable inhibition of the parathyroid secretion by the calcium carbonate ingestion was replaced by temporary "hyperparathyroidism" when the extract was simultaneously administered. The comparative changes in serum and urinary calcium reflect these alterations in parathyroid activity.

### THE CLEARANCE OF DIFFUSIBLE CALCIUM IN CLINICAL HYPERPARATHYROIDISM BEFORE AND AFTER REMOVAL OF THE ADENOMA

Only five patients (Group f) with normal renal function were studied (Table 6). Because of the variability of the data, each patient will be discussed separately.

*Patient Ri.* (48-year-old female). The calcium clearance of approximately 7.8 at a serum calcium of 13.2 mg.% (diff. Ca: 9.2) is below the normal range (Figs. 4, 6, 8) *when compared with the normal at comparable blood levels.* At a serum phosphorus of 2.0, the phosphorus clearance of 13.6 is definitely elevated.[22] Within 24 hours following removal of the adenoma, the serum calcium had fallen to 9.1 mg.% (diff. Ca: 6.3). The clearance of calcium was not sustained and decreased to the low value of 0.2. This should be contrasted with the increased clearance in the normal subjects following withdrawal of the PTE (Fig. 11).

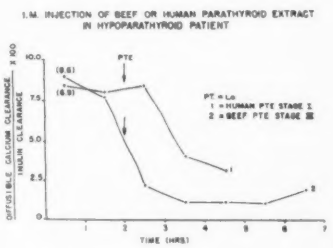


FIG. 9. The effect of a single intramuscular injection of human or beef parathyroid extract on the diffusible calcium clearance in a hypoparathyroid patient. Each curve represents a single experiment. The numbers in parenthesis refer to total serum calcium in mg.%.

*Patient Au.* (26-year-old male). This patient had only preoperative studies done. The calcium clearance of 4.2 at a serum calcium of 13.4 mg.% (diff. Ca: 8.7) was distinctly low.

*Patient Ca.* (35-year-old male). This patient had a preoperative clearance of calcium of 9.3. This could be considered a low normal value for a serum calcium of 14.4 mg.% (diff. Ca: 9.4). Four days postoperatively the serum calcium had decreased to 8.5 mg.%, and the phosphorus clearance from 65 to approximately 15. At this point the clearance of calcium was

4.5, considerably higher than would normally be observed at this calcium concentration.

*Patient Ba.* (55-year-old male). The calcium clearance was approximately 6.7 at a serum calcium concentration of 11.8 mg.% (diff. Ca: 7.6). This clearance was low when compared to those of the normal subjects at similar serum values (Figs. 4, 6, and 8). Forty hours postoperatively serum calcium had decreased to 7.9 mg.% (diff. Ca. 4.7), phosphorus clearance to 0.1, and calcium clearance to 4.4. These phosphorus and calcium clearances are consistent with a period of physiological hypoparathyroidism.

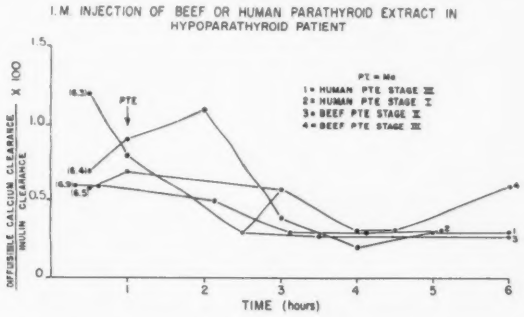


FIG. 10. The effect of a single intramuscular injection of human or beef parathyroid extract on the diffusible calcium clearance in a hypoparathyroid patient. Each curve represents a single experiment. The numbers in parenthesis refer to total serum calcium in mg.%.

Two months after surgery a calcium infusion was given to elevate the serum diffusible calcium to levels observed before operation. At a concentration of diffusible calcium of 7.6 mg.%, the clearance was 6.7. This value was comparable to that noted preoperatively. Four months later the clearance of calcium was very low (0.5), and the phosphorus clearance was almost as high as the preoperative value. We were unable to do further studies on this patient to investigate the possibility of recurrent hyperparathyroidism.

*Patient Bg.* (54-year-old male). This patient demonstrated distinctly low clearances (4.2 and 3.0) referable to his serum calcium of 11.4 mg.% (diff. Ca: 7.4) and 11.9 mg.% (diff. Ca: 7.7) prior to operation. Twelve to 15 hours after removal of the adenoma, his serum calcium had decreased to 9.7 mg.% (diff. Ca: 6.4). Despite this, the clearance of calcium *rose to* 5.0. In the period of 18-24 hours postoperatively, serum calcium was 9.3 mg.%, calcium clearance had decreased to 2.4, and phosphorus clearance was at its lowest level, 1.9.

**THE EFFECT OF PARATHYROIDECTOMY AND PARATHYROID EXTRACT ADMINISTRATION ON THE CLEARANCE OF CALCIUM IN THE DOG**

The calcium clearances illustrated in Figures 13 and 14 have been calculated from the concentration of total calcium in the serum. Subsequent ultrafiltration of the serum from these animals indicated that the clearance of diffusible calcium would be approximately 30-35 per cent higher. During the five-hour period following parathyroidectomy, the concentration of

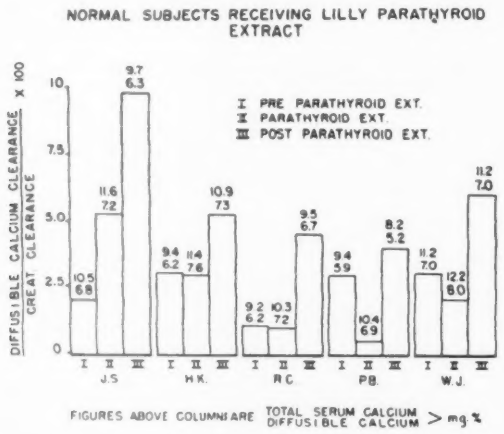


FIG. 11. The endogenous clearance of diffusible calcium, before, during, and after the administration of parathyroid extract (Lilly) for two or three days. Five subjects are represented. The figures above the columns are total serum calcium over the diffusible calcium in mg.%.

calcium in the serum decreased gradually from 9 to 8.5 mg.% (Fig. 13). Despite this fall in filtered load the calcium clearance rose sharply and remained above the control levels for the next five hours. No significant changes in serum calcium or phosphorus or their clearances occurred in two sham-operated dogs over the five-hour period after surgery.

Various stages of beef and human PTE were injected intramuscularly into four stable parathyroidectomized dogs maintained on high calcium diets without vitamin D. Calcium clearances were followed over a period of five-six hours after a single injection. The results are illustrated in Figure 14. Calcium clearance fell to a minimum approximately three hours after the injection. The greatest decreases were observed in the two animals with the highest initial clearances. These two dogs, despite their low serum calcium (7.3 and 4.0 mg.%), had initial clearances comparable to the six

dogs prior to parathyroidectomy (Fig. 13). The results of these dog experiments confirm those of Talmage, Kraintz, and Buchanan in the rat[15,16] and Buchanan, Kraintz, and Talmage in the mouse.[25] Martin, Mikkelson, and Jones, although not measuring clearances, have recently noted that parathyroidectomy in the dog causes a rise in calcium excretion.[26]

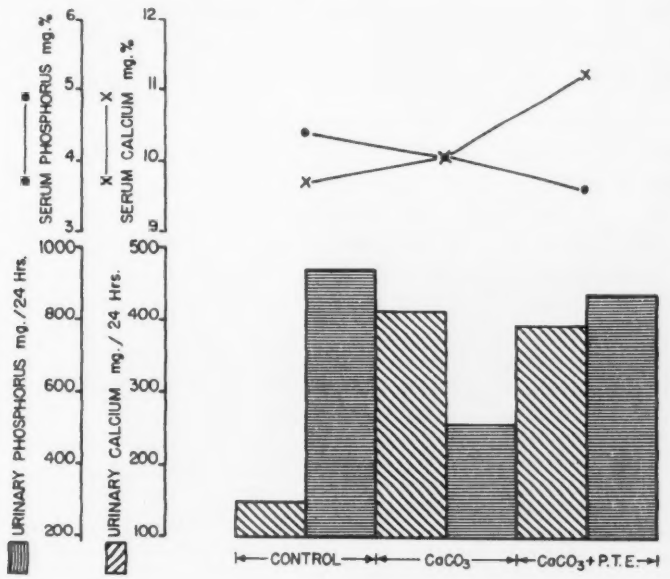


FIG. 12. The effect of calcium carbonate ingestion on the excretion of calcium and phosphorus and the serum concentration of these ions when the carbonate is administered with and without parathyroid extract (Lilly). The values represent the mean of four subjects.

## DISCUSSION

The excretion of calcium is a function of the filtered load of ionic and complexed calcium and the homeostatic factors regulating active tubular reabsorption. The results of the present investigation indicate that for any given level of dietary calcium and phosphorus or parathyroid activity, the greater the filtered load of calcium the greater was its renal clearance despite an absolute increase in the amount reabsorbed per minute (Figs. 3-6 and Table 3). Chen and Neuman[3] and Williamson and Freeman[37] made similar observations in the calcium-loaded dog. Jahan and Pitts[20] administered Lilly PTE to two normal dogs. As hypercalcemia developed and

the filtered load of calcium increased, the tubular reabsorption of calcium (mg./min.) greatly exceeded the rate observed when the animals were normocalcemic. These authors *correctly* attributed the increased reabsorption to the increased filtered load. It is not possible to conclude from their data, as has been done,[35] that parathyroid hormone caused an increased tubular reabsorption of calcium.

Assuming a normal rate of glomerular filtration and a constant per cent diffusible calcium, Talbot calculated the absolute rate of tubular reabsorption in normal subjects and patients with hypo- and hyperparathyroidism.[34] He used the term "$T_m$" to refer to absolute and not maximal rate of reabsorption. "Calcium $T_m$ is higher in patients with primary hyperparathyroidism (8.0 Gm. per $m^2$ per day) than in normal subjects (5.0 Gm. per $m^2$ per day) or patients with primary hypoparathyroidism (2.0 Gm. per $m^2$ per day)." From these calculations he concluded: "Doses of parathyroid extract act to boost the lowered serum calcium concentration toward normal, in part, by increasing the efficiency of calcium reabsorption by the renal tubules." Although his conclusion is probably correct, it could not be made by comparing tubular reabsorption at such widely differing filtered loads of calcium. His calculations simply showed that the absolute rate of calcium reabsorption (mg./min) was directly related to the concomitant serum calcium level and, therefore, to the filtered load. It is quite apparent that one cannot interpret the renal clearance of calcium in disease or altered physiological states without the realisation that an increased filtered load per se increases calcium clearance. Recently, Canary and Kyle[20] concluded that parathyroid hormone did not affect the tubular reabsorption of calcium because they found that the *per cent of the filtered load reabsorbed* in hyperparathyroidism was the same as in normal subjects. In interpreting their data, they did not take into consideration their observations that the filtered load of calcium in their hyperparathyroid patients was 10.3±0.8 mg./minute while in their normal subjects it was only 7.1±0.4 mg./minute. Actually, their data indicated that for a filtered load of 10.3 the patients had

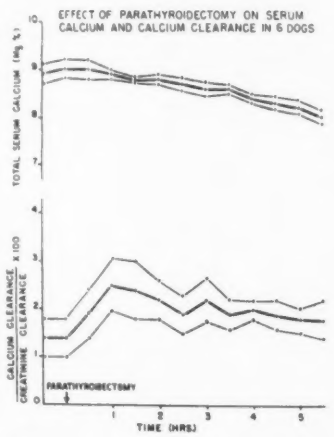
EFFECT OF PARATHYROIDECTOMY ON SERUM CALCIUM AND CALCIUM CLEARANCE IN 6 DOGS
TOTAL SERUM CALCIUM (mg %)
CALCIUM CLEARANCE / CREATININE CLEARANCE X 100
PARATHYROIDECTOMY
TIME (HRS)

FIG. 13. The effect of parathyroidectomy on the serum calcium and calcium clearance in six dogs. The curves represent the mean values and the standard deviation from the mean.

a *low* clearance of calcium. This was substantiated by the mean clearance of 3.6±0.3 ml./minute.

The results of the present investigation strongly suggest that for any given filtered load of calcium, regardless of the physiological state of calcium metabolism, the greater the level of parathyroid activity of circulating parathyroid hormone(s), the lower will be the renal clearance of diffusible calcium. These observations support the conclusions of Talmage and his associates[15,19,25] that the secretion of the parathyroid glands contributes homeostatically to the regulation of the tubular reabsorption of

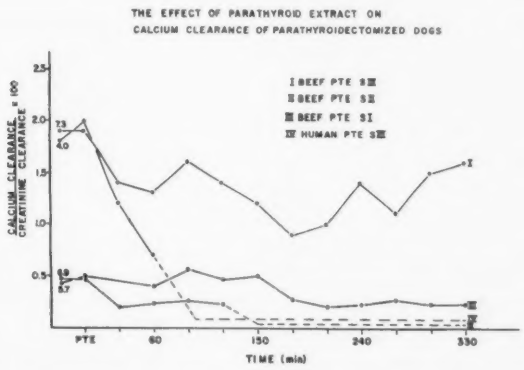


FIG. 14. The effect of beef and human parathyroid extract on the renal clearance of calcium in hypoparathyroid dogs. The dotted termination of two of the curves represents the estimated clearance values. The concentration of calcium in the urine of these samples was too low for accurate determination. The numbers adjacent to the beginning of each curve represent the total serum calcium concentration.

calcium. The summarized results contributing to this conclusion are as follows:

1. The endogenous clearance of calcium was lowered in normal and hypoparathyroid individuals by the administration of various types of human and beef PTE (Table 1, Table 4 (1-9), and Figs. 9 and 10).

2. Normal subjects had significantly higher calcium clearances on high calcium-low phosphorus diets ("physiological parathyroid hypofunction") than when on low calcium-high phosphorus diets ("physiological parathyroid hyperfunction") (Table 1).

3. The endogenous clearances of calcium were normal in two hypoparathyroid patients, despite hypocalcemia (Table 1, Bu. and Ma.), and the highest endogenous and post-calcium infusion clearances observed were found in a hypoparathyroid patient (Fig. 7).

4.The endogenous clearance of calcium increased acutely following PTE administration during the period of physiological hypoparathyroidism in normal subjects (Fig. 11), following accidental removal of the parathyroids (Table 4 (1) ), and after removal of a parathyroid adenoma (Table 6, patient Bg.).

5. The administration of Lilly PTE during calcium carbonate ingestion was not associated with an increase in calcium excretion even though serum calcium increased appreciably (Fig. 12).

6. The renal clearance of calcium was relatively low in patients with hyperparathyroidism (Table 6).

7. Parathyroidectomy in the dog resulted in an acute rise in the renal clearance of calcium despite a fall in the filtered load of calcium (Fig. 13).

8. Single injections of various preparations of beef and human PTE in the dog caused a decrease in the clearance of calcium (Fig. 14).

9. Acute administration of Lilly PTE in normal and hypoparathyroid subjects during a calcium infusion lowered the clearance of calcium for any given increment in the filtered load (Figs. 3-5).*

Certain aspects of the above summary require further comment. Although diets with a high Ca/P and a low Ca/P ratio can decrease and increase parathyroid activity,[7-9] respectively, it is not at all certain that the increase and decrease in calcium clearance accompanying these diets[10-12] (Table 1) are due to altered parathyroid activity per se. A slightly greater rise in serum calcium (filtered load) on the high calcium diet might also be a factor in increasing the clearance. Furthermore, phosphorus depletion causes a loss of osseous calcium and increased calcium excretion.[9-10] Whether this process is initiated at the skeletal or renal tubular levels is unknown. However, infusion of calcium into the subjects on the low calcium-high phosphorus diet (a procedure that immediately decreases parathyroid activity) inhibited the homeostatic reduction in the endogenous clearance of calcium (Fig. 8). In one recently completed experiment we have noted that the acute administration of Lilly PTE to a subject who had been on a high calcium-low phosphorus diet lowered his endogenous clearance to the level observed on the low calcium-high phosphorus diet.

The three patients with hypoparathyroidism studied in this investigation were chosen because two (Ma. and Bu.) had not been on vitamin D, and the dose (10,000 to 15,000 U.) taken by the third (La.) was thought to be

* The reason why the administration of PTE in three of the nine subjects (Fig. 6) did not lower the calcium clearance is not readily apparent. A possible explanation may be that the sensitive tubular mechanism affected by parathyroid hormone can be masked by the rapid rise in the filtered load of calcium.

below the therapeutic range necessary to treat hypoparathyroidism. Their endogenous clearances of calcium were normal-to-high. Most patients, however, with chronic untreated hypoparathyroidism (particularly the idiopathic type) have very low levels of calcium excretion. The low levels of filtered calcium certainly contribute to the hypocalciuria. It is possible that after many years of hypoparathyroidism they develop some additional homeostatic mechanism to enhance the reabsorption of calcium. Goldman and Bassett[30] infused calcium into three patients with untreated idiopathic hypoparathyroidism. Assuming a diffusible calcium of 65 per cent, the clearances in these three patients were markedly elevated at hypocalcemic and normocalcemic levels (10 cc., 9 cc., and 11 cc./min. at serum calciums

TABLE 7. THE RATE OF CALCIUM EXCRETION DURING A CALCIUM INFUSION EXTRAPOLATED TO A 24-HOUR LEVEL

| *Serum calcium during infusion* | *No. of Experiments* | *Urine calcium (mg. per 24 hours)* | | |
|---|---|---|---|---|
| | | *Mean* | *Low* | *High* |
| 10.0-10.9 | 6 | 1772 | 1087 | 2642 |
| 11.0-11.9 | 9 | 1901 | 863 | 2327 |
| 12.0-12.9 | 5 | 2126 | 700 | 3015 |
| 13.0-13.9 | 5 | 2168 | 1711 | 3190 |
| 14.0-14.9 | 3 | 1607 | 1056 | 2258 |

of 7.2, 7.9, and 10.8 mg.%, respectively). It is apparent that very high clearances of calcium can be observed in hypoparathyroid patients not receiving vitamin D. It has recently been suggested that the hypercalciuria seen in vitamin D-treated cases of hypoparathyroidism is due to a direct effect of vitamin D on the tubular reabsorption of calcium.[31] It seems more reasonable to conclude that vitamin D acting at the osseous level increases serum calcium, and, in the absence of the parathyroid glands, the clearance rises to abnormally high levels. In preliminary experiments we have been unable to detect an effect of vitamin D on the renal clearance of diffusible calcium in normal subjects on diets of varying calcium and phosphorus content.[32]

In some of our patients following removal of a parathyroid adenoma, the renal clearance of calcium did not increase during a period when acute temporary hypoparathyroidism was suspected (Table 6). This contrasted sharply with our normal subjects (Fig. 11). It seems probable that the marked depletion of skeletal calcium that is characteristic of these patients may, by an undisclosed mechanism, enhance the tubular reabsorption of calcium *independent of the level of parathyroid activity*. It is of interest

that when the rate of calcium excretion in our normal subjects receiving calcium infusions is extrapolated at different blood levels to a 24-hour excretion, the amount is considerably in excess of that usually encountered in clinical hypercalcemia (Table 7). This suggests that in chronic hypercalcemic states, usually with osseous depletion of calcium, the tubular reabsorption may be somewhat greater for a given serum calcium level than following an acute infusion of calcium. However, the clearance of calcium is usually lower for any degree of hypercalcemia in hyperparathyroidism than in other hypercalcemic disorders.[88]*

The exact mechanism(s) by which the secretion of the parathyroids decreases the renal clearance of calcium is unknown. It is conceivable that the renal effect is not due to the direct action of the hormone at the renal tubular level but actually is secondary to its acute effects on osseous metabolism. However, in both dogs and humans we observed a fall in calcium clearance without a noticeable skeletal effect (a rise in serum calcium) (Table 4 (1-9) ). Unfortunately, the results of infusing parathyroid extract into one renal artery of the dog have to date proved too erratic to interpret.

SUMMARY

1. The effect of calcium infusions on the renal clearance of diffusible calcium has been investigated in normal and hypoparathyroid subjects.

2. An increase in the filtered load of calcium increased the renal clearance of diffusible calcium in all subjects.

3. For any given filtered load of calcium, the greater the level of parathyroid activity, the lower the clearance of calcium.

4. Experimental and clinical states of hyperparathyroidism were associated with a relative decrease in calcium clearance.

5. Experimental and clinical states of hypoparathyroidism were associated with a relative increase in the clearance of calcium.

6. Acute administration of parathyroid extract to hypoparathyroid humans and dogs decreased the renal clearance of calcium.

7. The secretion of the parathyroid glands contributes to the homeostatic regulation of the tubular reabsorption of calcium.

* In a review of 20 recent articles on primary hyperparathyroidism selected at random, 46 cases without renal insufficiency were encountered. Seventeen of these had normal levels of calcium excretion (<200 mg./24 hours). The serum calciums in these 17 ranged from 10.5-18.0 mg.%. To conserve space these references have not been included in the bibliography but may be obtained from the authors on request.

ADDENDUM

Since completion of the manuscript, we have noted the article by Crawford, Loughridge, Milne, and Scribner[32] on organic acid excretion after calcium gluconate infusions. These investigators found that 68-80 per cent of the infused gluconate was excreted as the unaltered anion in the urine. Although they concluded that the gluconate was excreted predominantly as sodium and potassium salts, their findings emphasize the need to compare the effects of chloride and gluconate salts of calcium on the renal clearance of calcium in paired studies.

ACKNOWLEDGMENT

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# LACK OF CORRELATION BETWEEN GOUT AND THE INCORPORATION OF ISOTOPIC FORMATE INTO URIC ACID

Certain reports[1–4] have indicated that patients with gout incorporate the isotopic precursors of the purine ring into urinary uric acid to a greater extent than do non-gouty individuals. The possibility of utilizing this difference as a diagnostic test for gout has been explored. Carbon-14 labeled sodium formate was given orally or intravenously to 10 patients known to have gout, to 3 with hyperuricemia but no gouty symptoms, and to 16 non-gouty subjects without elevated serum uric acid levels. Three patients who had received oral formate were also given intravenous doses after the urinary uric acid specific radioactivity had become negligible. No correlation between the incorporation of isotope into urinary uric acid and the presence of gout or hyperuricemia was noted.

## METHODS

For oral administration doses were prepared that contained 10$\mu$c. (0.6 mg.) of sodium formate-$C^{14}$ mixed with 400 mg. of lactose. Enough labeled sodium formate for several doses was dissolved in a small quantity of water, added to dry lactose, and, after drying in a vacuum oven, the mixture was ground in a mortar and weighed into gelatin capsules on an analytical balance. The specific radioactivities of the mixture were found to be constant between capsules, and the radioactivity was shown to be virtually all formate by specific oxidation with mercuric ion.[5] For intravenous administration sodium formate-$C^{14}$ was dissolved in a vial of sterile physiological saline to a concentration of 10$\mu$c. (0.6 mg.) in 3 ml. and autoclaved.

For experiments with oral dosage hospital patients who had just voided were given a capsule at 8:00 a.m. Breakfast was withheld until 10:00 a.m. and urine was collected until 4:00 p.m. The intravenous experiments were identical except that the dose contained no lactose.

After removal of a 5 ml. aliquot from the eight-hour urine specimen for uric acid determination the bulk of the remaining uric acid was precipitated as ammonium urate.[6] After the crude precipitate had settled it was concentrated by centrifugation, converted to uric acid with 3 ml. of 6 N hydrochloric acid, and washed with 3 ml. of

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TABLE 1. INCORPORATION INTO URINARY URIC ACID OF ISOTOPIC FORMATE GIVEN ORALLY

| *Patient* | *Diagnoses* | *Serum uric acid* | | *Excretion in 8 hrs.* | *Uric acid specific radioactivity* | *Total excreted radioactivity in uric acid* |
|---|---|---|---|---|---|---|
| | | *Uricase mg. %* | *Folin mg. %* | *mg.* | *cts m/mg.* | *cts* |
| CONTROLS | | | | | | |
| A.B. | Intra-atrial septal defect | 6.9 | 4.2 | 140 | 177 | 24,800 |
| E.C. | Traumatic myositis | 4.2 | 3.7<br>5.0 | 159 | 156 | 24,800 |
| M.G. | Chronic lymphatic leukemia | 5.2 | 3.7<br>5.0, 4.5, 4.6 | 194 | 113 | 21,900 |
| M.B. | Essential hypertension | 4.8 | 4.5<br>1.6, 1.5, 4.8<br>6.5, 5.1, 5.3,<br>4.7, 7.0 | 128 | 110 | 14,100 |
| T.R. | Urticaria | — | — | 190 | 72 | 13,700 |
| T.C. | Essential hypertension, myocardial infarct | 6.2<br>7.6 | 5.8<br>6.5 | 57 | 177 | 11,200 |
| R.A. | Chronic lymphatic leukemia | 5.3 | 4.9<br>4.6 | 162 | 44 | 7,130 |

| | | | | | | |
|---|---|---|---|---|---|---|
| GOUTS | | | | | | |
| S.F. | Hyperuricemia, nephrolithiasis, chronic bronchitis No arthritis | 5.5 | 5.5<br>7.7, 8.5, 8.3<br>6.0, 6.5 | 209 | 93 | 19,400 |
| W.S. | Gout | 5.8 | 5.7<br>7.7, 7.6, 20.0<br>11.2, 11.6, 10.8,<br>5.4, 8.0, 8.0<br>8.5, 8.0 | 131 | 105 | 13,800 |
| G.C. | Gout, diabetes, sarcoma | 7.4 | 8.2<br>7.0, 6.0, 6.1<br>6.8, 5.3 | 170 | 76 | 12,900 |
| M.S. | Pylolithiasis, urate stone, no arthritis Hyperuricemia? | 7.8 | 7.0<br>5.1, 4.5, 4.9<br>7.0, 7.3 | 204 | 61 | 12,200 |
| S.M. | Gout, tophi | 10.6 | 8.5<br>8.0, 9.5, 5.5<br>12.4, 10.8 | 182 | 52 | 9,460 |
| L.K. | Gout, Glomer-ulonephritis | 10.3 | 6.3<br>10.9, 7.2, 7.8,<br>5.2, 8.5, 7.6,<br>5.8, 6.3 | 206 | 27 | 5,360 |

water. It was then dissolved in a minimal quantity of N lithium hydroxide. After centrifuging to remove insoluble impurities, the supernatant was filtered by suction through a 0.5 by 5 cm. column of a half and half mixture of cellulose and charcoal. The column was poured on 2 cm. of pure cellulose to prevent the escape of charcoal into the filtrate. Uric acid was finally precipitated by acidification of the filtrate with glacial acetic acid. The centrifuge tube containing the sample was placed in boiling water for five minutes to convert a very flocculent precipitate to granular crystals. The product was twice recrystallized by solution in lithium hydroxide and precipitated with acetic acid. The purified uric acid was washed twice each in water, alcohol, and ether, and dried in a vacuum oven. The specific radioactivity of the recrystallized material was identical to that first precipitated with acetic acid. A high state of purity was confirmed spectrophotometrically.

Determination of the specific radioactivity was performed by proportional gas counting after sealed tube combustion.[7] Uric acid determinations of urine and some of serum were performed by the photometric uricase method of Kalckar[8] as modified by Praetorius.[9] Prepared tubes of buffered uricase* were used for most determinations. Serum levels were also performed by the hospital clinical chemistry laboratory with the Folin colorimetric method.[10]

The diagnosis of gout was established by history and the presence of arthritis typical of gout. It was confirmed in all but one instance by at least one serum uric acid level above 8 mg. per cent. The exception (A.C. Table 2) had a ten-year history of attacks of acute arthritis typical of gout and relieved by colchicine. Two gout patients had tophi. Three patients had elevated serum uric acid levels but no arthritis. Two of these had nephrolithiasis and the highest serum uric acid of one (M.S., Table 1) was 7.8 mg.%. Criteria for the absence of gout in the control series were: failure to elicit a history of arthritis resembling gout, absence of physical signs, and serum uric acid levels below 8 mg.

## RESULTS

Table 1 gives the data on the patients who received oral doses and Table 2 gives the data on those getting labeled formate intravenously. The two patients with hyperuricemia and the one with questionable hyperuricemia and urate stone are listed with the gouts. The specific radioactivities of the uric acid isolated from the oral dose group were considerably higher than those in the intravenous group. Serum uric acid levels are given for each patient. The values first listed for each patient in Table 1 are those obtained from blood taken on the day of the study, and where the uricase method was employed, the Folin result given on the same line was obtained on the same serum. In general the uricase levels were slightly higher. The other values (all Folin) were obtained on various other occasions during the patients' hospitalizations. Uricase determinations were not performed on the sera of most of the patients of Table 2 and those few that were done

*Determatube-U, Worthington Biochemical Corporation, Freehold, New Jersey.

TABLE 2. INCORPORATION INTO URINARY URIC ACID OF ISOTOPIC FORMATE GIVEN INTRAVENOUSLY

| *Patient* | *Diagnoses* | *Serum uric acid mg. %* | *Excretion in 8 hrs. mg.* | *Uric acid specific radioactivity cts/m/mg.* | *Total excreted radioactivity in uric acid cts* |
|---|---|---|---|---|---|
| CONTROLS | | | | | |
| W.M. | Infectious mononucleosis | 4.5, 4.8 | 335 | 57 | 19,100 |
| J.Z. | Duodenal ulcer | 6.3 | 354 | 40 | 14,200 |
| J.D. | Hemorrhagic cyst of thyroid | 4.4, 3.9 | 272 | 51 | 13,900 |
| E.C. | Traumatic myositis | 3.7, 5.0, 4.2 | 194 | 48 | 9,310 |
| M.G. | Chronic lymphatic leukemia | 3.7, 5.0, 4.5 4.6, 5.2 | 182 | 33 | 6,000 |
| A.Q. | Contact dermatitis | 6.2 | 236 | 25 | 5,900 |
| M.T. | Undiagnosed renal disease, elevated BUN | 6.3, 6.0, 4.2 4.8, 4.6 | 264 | 20 | 5,280 |
| V.T. | Duodenal ulcer | 6.0, 4.3, 6.9 | 87 | 41 | 3,570 |
| M.H. | Hypertensive cardiovascular disease, cerebrovascular accident | 3.8 | 160 | 21 | 3,360 |
| GOUTS | | | | | |
| G.O. | Gout | 12.5, 7.1, 9.0 7.5, 8.0, 7.5, 6.8, 6.8, 8.5, 11.0, 11.3 | 268 | 32 | 8,580 |
| G.C. | Gout, diabetes sarcoma | 8.2, 7.0, 6.0 6.1, 6.8, 6.3, 7.4 | 211 | 39 | 8,230 |
| A.K. | Gout, arteriosclerotic and rheumatic heart disease | 9.0,6.1 | 147 | 51 | 7,500 |
| F.F. | Gout, tophi | 7.0, 7.3, 4.6. 5.3, 7.4, 6.5, 10.5, 6.5, 7.0 | 298 | 25.0 | 7,450 |
| F.L. | Hyperuricemia, senile cataract, No arthritis | 11.0, 9.3, 9.3 | — | 18.8 | —— |
| A.C. | Gout, chronic cellulitis | 6.5, 4.7 | 240 | 18.2 | 4,370 |
| J.F. | Gout, cerebrovascular accident | 12.5, 5.8 | — | 11.0 | —— |
| J.G. | Gout, hypertensive cardiovascular disease | 5.5, 9.2 | 268 | 6.85 | 1,840 |

are grouped with the results from the clinical chemistry laboratory. The uric acid content of each eight-hour urine is listed in each table.

In the oral dose series five non-gouty subjects showed a higher specific radioactivity of uric acid than any gouty subject, and the patient with the lowest value had gout. There was a four-fold spread of values among the non-gouty patients as well as among the gouty patients. When the total

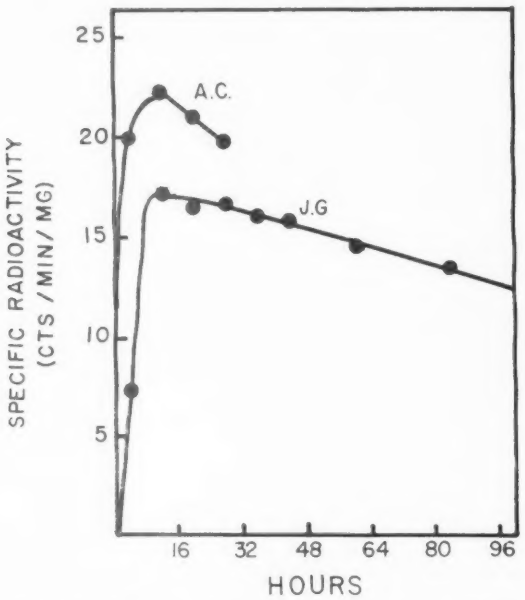


FIG. 1. Specific radioactivity of urinary uric acid from two patients with gout who received 10 $\mu$c. of formate-$C^{14}$ intravenously.

counts in excreted uric acid were computed, the three highest values fell in the control group, and again the patient with the lowest value had gout.

The correlation between uric acid specific radioactivity and gout was equally as poor in the patients who received the labeled formate by vein. When the data were computed on the basis of total counts in excreted uric acid, the correlation was no better. Again the patient with the lowest incorporation by either method of computation had gout. Because these studies covered only an eight-hour period, urine from this patient (J. G.) and one other (A. C.) was collected over a longer period. The results are shown in Figure 1. In both patients the peak specific radioactivity occurred

in the sample collected between 8 and 16 hours but in neither did it rise to the level seen in the majority of the non-gouty control patients.

## DISCUSSION

The work of Benedict *et al.*,[1,2] who administered $N^{15}$ glycine, seemed to indicate that at least some patients with gout incorporate greater quantities of isotope into uric acid than do normal subjects. However, two of the four gouty subjects studied could not be distinguished from the two normal controls. Wyngaarden[4] postulated that the large chemical dose of glycine used might have masked the difference in incorporation, and with tracer doses of $C^{14}$ glycine he found a high correlation between the extent of incorporation and gout. Each of seven gouty patients (one of these with asymptomatic hyperuricemia) incorporated more isotope into urinary uric acid than did three control subjects. Spillman[3] gave $C^{14}$ formate to two patients with gout and two normal controls. The gouty patients had a six-fold greater peak specific radioactivity in the urinary uric acid than did the controls. He also found, as had Wyngaarden, that the peak specific radioactivity occurred much sooner in the patients with gout. These findings led us to believe that a short-term collection of urine would be more useful diagnostically than a longer time study with multiple samples. It would certainly be more convenient.

A subsequent report by Wyngaarden[11] includes data on two gouty subjects that did not incorporate more isotopic glycine than did the controls, and an accompanying paper by Seegmiller *et al.*[12] reported that two subjects without gout had a higher degree of incorporation than previously reported control subjects.

Other laboratories have reported similar studies which involved only one or two patients with one control in each study. In both experiments with a single gouty patient[13,14] the degree of incorporation was indistinguishable from that of the control. In the study involving two gouty subjects[15] the peak isotope content of uric acid in the control fell between those of the gouty patients.

In all of the reports that we have seen, 26 gouty patients (including asymptomatic hyperuricemia) and 14 control subjects have been studied. Because different compounds and somewhat different techniques were employed, it is difficult to reduce all data to a common basis for comparison. However, only 14 of the gouty subjects incorporated significantly more isotope than the control subjects of the same study.

Our study is in essential agreement with this composite picture except that we found the highest incorporation in patients of the control group.

When the data are considered in the light of information already available, it appears that there is great variability in the extent of incorporation of isotopic precursors into urinary uric acid in gouty as well as in non-gouty subjects. It would appear that a very extensive study and careful statistical analysis would be necessary if any significant difference between gouts and normals were to be uncovered by this type of study.

It is beyond the scope of this paper to review the evidence that gout is related to the overproduction of uric acid by a "shunt" mechanism,[16] but the results of isotopic studies have been given as the chief evidence to support this hypothesis. If all the studies cited are considered, the evidence in support of the overproduction theory seems weak indeed, and it is possible that the results of individual studies that have favored the overproduction theory occurred only fortuitously. The view that the hyperuricemia of gout may be related to a defect in the renal excretion of uric acid has been considerably strengthened by a report by Nugent and Tyler.[17] These workers pointed out that in earlier clearance studies serum levels of uric acid were not at the same levels in the gouty patients and in the controls. When they maintained elevated levels in the control subjects by dietary supplementation with ribose nucleic acid, the uric acid clearance rates were much higher in the non-gouty subjects than in those with the disorder. Latham and Rodnan[18] have very recently confirmed these findings by infusing lithium or sodium urate.

## SUMMARY

Isotopically labeled formate was administered orally or intravenously to patients with gout or asymptomatic hyperuricemia and to control patients. Urinary uric acid was isolated from urine voided during the eight-hour period following the dose. No correlation between the presence of the disease and the specific radioactivity of the uric acid was found.

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# EXPERIMENTAL PYELONEPHRITIS. IX. THE BACTERIOLOGICAL COURSE AND MORPHOLOGICAL CONSEQUENCES OF STAPHYLOCOCCAL PYELONEPHRITIS IN THE RAT, WITH CONSIDERATION OF THE SPECIFICITY OF THE PATHOLOGICAL CHANGES OBSERVED¶

## INTRODUCTION

Although coliform bacteria are the organisms most commonly recovered from the urine of patients with pyelonephritis, they seldom cause infection in the absence of some kind of renal injury.[1] These bacteria are not suitable, therefore, for experiments designed to study alterations of structure and function attributable to infection alone. For these purposes it would seem preferable to employ microorganisms capable of establishing infection in normal kidneys, e.g. staphylococci.

The present series of experiments was carried out in the rat since renal physiology and pathology have been studied extensively in this animal. Other investigations of staphylococcal pyelonephritis have been performed in mice or rabbits.[2,3,4] Such species were considered unsatisfactory for these investigations since they are not well suited for studies of renal physiology and since the mortality is appreciable following production of extensive pyelonephritis with virulent staphylococci.

This report describes the bacteriological and morphological course of staphylococcal pyelonephritis in normal rats. The alterations in renal function produced by this infection will be described in the paper which follows.

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## MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200 to 250 grams served as the experimental animal in all studies.

The *Staphylococcus aureus* "Giorgio" was the test organism. It is coagulase-positive, hemolytic, and has been described in full by Smith and Dubos.[4] This organism has been used in experiments previously reported from this laboratory.[5]

The techniques employed for determining the number of bacteria in culture media and tissues of the rat have been given in detail in previous pulications.[6] One half of each kidney was taken for culture and the remainder used for histological study. Urine was cultured by streaking a loopful on a blood agar plate. All inocula were obtained from overnight cultures prepared in beef heart infusion broth.

The tail vein of the rat was the site of intravenous injections.

Histological studies were carried out on 3-4 slides obtained from a longitudinal hemisection of the kidney.

## RESULTS

### *Production of pyelonephritis following intravenous injection of staphylococci*

The injection of $10^8$ staphylococci resulted in the production of renal infection in approximately 100 per cent of animals. The course of this infection is shown in Table 1. Urine cultures did not become positive until 24 hours after inoculation at a time when bacterial multiplication could be detected in the kidney. Bacteremia persisted for two to four days. Approximately 10-20 per cent of animals died at intervals from two weeks to three months following injection. At autopsy these animals had extensive bilateral renal infection.

### *Size of inoculum necessary to produce renal infection*

The inoculation of $10^7$ organisms into ten rats resulted in renal infection at one week in only one animal. Both kidneys of this rat were heavily infected, and the urine contained a large number of bacteria. One other kidney contained 2,000 organisms and the remainder less than 100. All blood cultures were sterile. All urine cultures were sterile save for the sample obtained from the infected animal.

### *Course of renal infection*

Reference to Table 1 reveals that there was a natural tendency for the pyelonephritis to subside. Although occasional kidneys were found to be sterile or contain low numbers of organisms between 4 and 12 weeks, it was not until 15-22 weeks after inoculation that the majority of kidneys had become sterile. Starting 24 hours after the injection of organisms, the

TABLE 1. THE COURSE OF RENAL INFECTION FOLLOWING THE INTRAVENOUS INJECTION OF $10^8$ STAPHYLOCOCCI IN NORMAL RATS

| *Time interval* | *Kidney* | | *Blood* | *Urine* |
|---|---|---|---|---|
| | *Rt.* | *Lt.* | | |
| 2 hrs. | 18,000* | 21,000 | + | 0 |
| | 21,000 | 22,000 | + | 0 |
| | 22,000 | 18,000 | + | 0 |
| | 24,000 | 1,000 | + | 0 |
| | 31,000 | 73,000 | + | 0 |
| | 26,000 | 11,000 | + | 0 |
| 6 hrs. | 19,000 | 20,000 | | 0 |
| | 21,000 | 18,000 | | 0 |
| | 20,000 | 22,000 | | 0 |
| | 23,000 | 14,000 | + | 0 |
| | 24,000 | 37,000 | + | 0 |
| | 51,000 | 21,000 | + | 0 |
| 1 day | > 1,000,000 | > 1,000,000 | + | + |
| | 97,000 | > 100,000 | + | + |
| | > 100,000 | > 100,000 | + | + |
| | > 1,000,000 | > 1,000,000 | + | + |
| | 28,000 | 2,530 | + | 0 |
| | > 100,000 | 83,000 | + | 0 |
| 2 days | > 1,000,000 | > 1,000,000 | 0 | + |
| | 20,000 | > 1,000,000 | 0 | + |
| | 11,000 | > 1,000,000 | 0 | + |
| | > 1,000,000 | > 1,000,000 | + | + |
| | 53,000 | > 1,000,000 | + | + |
| | 2,330 | > 1,000,000 | 0 | + |
| 4 days | 39,000 | > 1,000,000 | + | + |
| | > 1,000,000 | > 1,000,000 | + | + |
| | > 1,000,000 | > 100,000 | + | + |
| | 0 | > 1,000,000 | + | + |
| | > 1,000,000 | > 1,000,000 | + | + |
| 1 week | > 1,000,000 | > 100,000 | 0 | + |
| | > 1,000,000 | > 1,000,000 | 0 | + |
| | > 100,000 | > 100,000 | 0 | |
| | > 1,000,000 | 37,000 | 0 | + |
| | > 1,000,000 | > 1,000,000 | 0 | + |
| | > 1,000,000 | > 100,000 | 0 | + |
| 2 weeks | > 100,000 | 100,000 | | |
| | 100,000 | > 100,000 | | |
| | 500,000 | 90 | | |
| 4 weeks | > 100,000 | 90 | | |
| | | 0 | | |
| | > 100,000 | > 100,000 | | |
| 6 weeks | > 100,000 | 4,000 | | |
| | > 1,000,000 | 0 | | |
| | 0 | 0 | | |
| 8 weeks | > 1,000,000 | > 1,000,000 | | |
| | > 100,000 | 100,000 | | |
| | > 100,000 | > 1,000,000 | | |

TABLE 1. (CONTINUED)

| *Time interval* | *Kidney* | | *Blood* | *Urine* |
|---|---|---|---|---|
| | *Rt.* | *Lt.* | | |
| 11-12 weeks | 0 | 0 | | |
| | > 100,000 | > 1,000,000 | | |
| | > 1,000,000 | > 1,000,000 | | |
| | > 1,000,000 | > 1,000,000 | | |
| | 11,000 | 0 | | |
| 15-18 weeks | 0 | 0 | | |
| | 0 | 0 | | |
| | > 1,000,000 | > 1,000,000 | | |
| | 0 | 0 | | |
| | 0 | 0 | | |
| | 0 | 0 | | |
| | 0 | 0 | | |
| | 0 | 0 | 0 | 0 |
| 22 weeks | 11,000 | 0 | + | + |
| | > 1,000,000 | > 1,000,000 | 0 | + |
| | 0 | 0 | 0 | 0 |
| | 12,000 | 9,000 | 0 | + |
| | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | > 100,000 | 0 | 0 | |
| 30 weeks | 0 | 60 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | > 100,000 | 60,000 | 0 | + |
| | 2,000 | > 100,000 | 0 | + |
| | 30,000 | 30,000 | 0 | + |
| | 0 | 0 | 0 | 0 |
| | 790 | 0 | 0 | 0 |
| | > 100,000 | 0 | 0 | + |
| | 0 | 0 | 0 | 0 |
| | 0 | 700 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | 20 | 10 | 0 | + |
| | 0 | 0 | 0 | + |
| | 0 | 30,000 | 0 | + |
| | 25,000 | 0 | 0 | + |
| | 6,000 | 0 | 0 | + |
| | 0 | 620 | 0 | + |
| | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| 51 weeks | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 |

*Colonies staphylococci per one-half kidney.

general rule was for the urine to contain staphylococci at a time the kidney contained staphylococci.

*Inoculation of staphylococci into bladder urine*

The urinary bladder of six rats was exposed through a midline abdominal incision and the bladder urine inoculated with $10^8$ staphylococci in a volume of 0.25 ml. of broth culture medium. The kidneys and ureters were not exposed or manipulated in any way. After an interval of four days the animals were examined. No gross abnormalities were seen in the kidneys or bladder. As shown in Table 2, significant numbers of bacteria were re-

TABLE 2. BACTERIOLOGICAL STUDY FOUR DAYS AFTER INOCULATION OF $10^8$ STAPHYLOCOCCI INTO BLADDER URINE OF NORMAL RATS

| | *Kidney* | | *Urine* | *Blood* |
|---|---|---|---|---|
| | *Rt.* | *Lt.* | | |
| 1. | 0 | 10* | 0 | 0 |
| 2. | 0 | 0 | 0 | 0 |
| 3. | 0 | 0 | | 0 |
| 4. | 1,600 | 15,000 | + | 0 |
| 5. | 300,000 | 4,000 | + | 0 |
| 6. | 0 | 0 | + | 0 |

*Colonies staphylococci per one-half kidney.

covered from four kidney cultures of two animals, although only one of these kidneys contained 300,000 viable units, a number indicative of infection. It is evident that staphylococci can make their way from bladder urine to the kidney in a normal animal.

*Pathology of staphylococcal pyelonephritis*
*Two hours—24 hours*

The earliest change detectable in the kidney was marked hyaline droplet formation in renal tubular epithelial cells located primarily in the cortex. Most tubules contained large droplets similar to those described by Oliver[7] and Sheehan and Davis.[8] They were seen up to one week after the injection of organisms but not to any significant degree after that time.

*Two days—1 week*

During this interval, foci of acute inflammation became visible both on gross and microscopic examination. The changes conformed with those described by others as occurring in mice and rabbits at similar intervals of time following the intravenous inoculation of staphylococci.[2,4,9]

The most prominent finding was pus-filled tubules, and regions of tubular destruction with abscess formation. These changes were confined to wedge-shaped areas, the apices of which were located in the papilla (Figs. 1, 2). Often three or four such wedges could be seen in one kidney, separated by normal renal tissue. Papillary involvement was sometimes so severe as to produce visible necrosis of a section of the papilla (Fig. 3). The limitation of areas of inflammation to discrete wedges was striking, resembling the infections produced in this laboratory previously by the inoculation of bacteria directly into the renal medulla.[5,10] Microscopically, the lesions were characterized by dense polymorphonuclear infiltration within and around cortical and medullary tubules (Fig. 4). Abscess formation within the wedges was accompanied by the loss of recognizable elements of renal structure. Isolated abscesses were occasionally seen in the medulla, surrounded by normal tissue, and encased in a thick wall of inflammatory cellular exudate. Colloid casts were sometimes seen in tubules at the corticomedullary junction surrounded by polymorphonuclear leucocytes. No abnormalities were seen in glomeruli or large or small blood vessels.

*Two weeks—1 year*

At two weeks the interstitial inflammatory cellular exudate was composed of polymorphonuclear leucocytes and lymphocytes. At four weeks only a few polymorphs could still be seen. Within distended tubules at four weeks, however, polymorphonuclear leucocytes persisted in large numbers. From this time on there was progressive atrophy of tubules within the areas of suppuration with consequent crowding of glomeruli (Fig. 5). Condensation of connective tissue in association with atrophic tubules around normal glomeruli presented a picture similar to periglomerular fibrosis. These microscopic changes were accompanied by gross depression and contraction of areas previously the site of bulging suppuration (Fig. 6). Abscesses in early stages of development were not seen in any kidneys containing areas of resolving inflammation. No glomerular or vascular abnormalities were seen in any of the kidneys examined.

A lesion often considered characteristic of healed pyelonephritis in man, which was observed in rabbits by Mallory, Crane, and Edwards, is that of colloid cast formation within dilated tubules.[11] These casts were encountered only twice in the present study and each time to a slight degree. These animals were examined 1 and 12 weeks after infection.

A peculiar lesion noted in one rat was extreme cystic dilatation of the medullary portion of a group of nephrons within an area of healed pyelonephritis (Fig. 7). This cyst formation is most likely a result of tubular

obstruction by scar tissue in the medulla and resembles the cysts produced by mechanical medullary obstruction in the rabbit.[12]

Acute polymorphonuclear leucocytic infiltration of the renal pelvis was frequently encountered from 6 to 30 weeks after inoculation of organisms (Fig. 8). These lesions were not seen in kidneys taken prior to six weeks or one year after infection. The occurrence of similar lesions in mice is mentioned by Gray *et al.*[9]

*Correlation of histology with bacteriological findings*

Animals examined between one day and one week after inoculation of organisms were virtually all found to have pyelonephritis histologically and bacteriologically. At two and six weeks sterile kidneys were encountered in which there was no morphological evidence of prior infection. After six weeks, however, almost all of the sterile kidneys demonstrated unequivocal morphological evidence of previous infection thereby indicating that healing had occurred.

The lesion of active (polymorphonuclear exudate) pyelitis was first encountered six weeks after injection. Between six and 22 weeks over half the kidneys which contained viable staphylococci had this microscopic lesion. At 30 weeks all kidneys which were positive on culture had active pyelitis except two. The finding of active pyelitis microscopically was associated in all instances with the recovery of staphylococci from the kidney. All but two kidneys with active pyelitis had, in addition, evidence of cortical and medullary scars.

*Extent of morphological damage*

It was very difficult to estimate the extent of renal damage upon either gross or microscopic examination of these kidneys due to the patchy nature of the inflammation. By gross inspection one could often detect large scars which might not appear in the microscopic section. Conversely, papillae which appeared normal on gross examination might reveal large areas of scarring microscopically. The only certain way of not missing morphological damage would be by serially sectioning whole kidneys. By the techniques used, however, scars might easily have been missed on both gross and microscopic examination. Despite these difficulties morphological evidence of infection was seen in most kidneys.

It was difficult to compare the extent of damage in the cortex with that in the medulla. It did appear, nevertheless, particularly during the early stages of the infection, that relative to its size, the papilla contained more inflammatory exudate than the cortex (Figs. 2, 9).

*"Pyelonephritic" lesions seen during the course of sterile hydronephrosis in the rat*

In an attempt to distinguish the pathological changes, described by others, of pyelonephritis in the presence of obstruction from those changes due to pyelonephritis alone, a group of six rats was examined three weeks after ligation and division of the left ureter. Marked hydronephrosis was evident. There was no gross evidence of infection, and culture of one half the obstructed kidneys was sterile. Despite the absence of infection, a number of lesions seen in these kidneys bore a remarkable resemblance to those described with staphylococcal infection in this report and in the study of coliform infection of the obstructed rabbit kidney by Mallory, Crane, and Edwards.[11]

Numerous examples were seen of dilated tubules filled with polymorphonuclear leucocytes and nuclear debris (Fig. 10). The similarity between these lesions and those which were seen in the early stages of staphylococcal pyelonephritis is evident (Fig. 4). Such tubular lesions have been described previously by Helmholtz and Field in the rabbit with hydronephrosis.[12] There was inflammatory exudate scattered through the interstitial tissues (Fig. 11), and dilated tubules filled with colloid casts were also seen (Fig. 12). Formation of colloid casts was more pronounced in these hydronephrotic kidneys than at any stage examined during the course of staphylococcal pyelonephritis. It was also possible in these kidneys to identify glomerular crowding (Fig. 13) and glomeruli in various stages of hyalinization (Figs. 14, 15).

## DISCUSSION

The rat seems to be as susceptible to renal infection with virulent staphylococci as the mouse and rabbit, but, in contrast to these species, the attendant mortality is negligible. A large number of bacteria is necessary to produce hematogenous pyelonephritis in the rat since reducing the inoculum by one log resulted in a sharp decrease in the percentage of animals infected. Despite the ability of staphylococci to infect normal kidneys, the natural tendency of the infection was toward healing, most kidneys being sterile 5-22 weeks after inoculation.

It is well known that the concentration of bacteria in blood is much higher immediately after intravenous injection than it is later, yet no bacteria were recovered in the urine up to six hours after injection. It was only when bacterial multiplication was detected in the kidney that these organisms could be recovered from the urine. It is evident that staphy-

lococci are not excreted into the urine by normal kidneys. Positive urine cultures later in the course of the infection occurred in those animals whose kidneys still contained culturable organisms in all but one instance, and since hemisections of the kidney were examined histologically, it is possible that a localized focus of infection was present in the single exception. The kidneys of animals with negative urine cultures were sterile save for three kidneys which contained 60, 700, and 790 bacteria.

The lesions of staphylococcal pyelonephritis in the rat kidney were similar to those previously described in the mouse and rabbit and, in addition, resembled those produced by *E. coli* in a kidney with intrarenal hydronephrosis.[5,6,10] The areas of suppuration were wedge-shaped with the apex in the papilla.

Particular attention was directed to the extent of damage produced in the renal papilla as compared with cortex in this infection. The impression was gained that *proportional to its size,* the papilla was more extensively involved by the process of inflammation than was the cortex. These observations will be cited in considering the alterations of renal function which result from this infection and are particularly relevant in comparing the functional effect of pyelonephritis in an animal without a well-formed papilla (dog) with its effect in the rat or man.

There did not appear to be any tendency for lateral spread of infection within the kidney. This is remarkable in view of the ability of the staphylococcus to infect the normal kidney and has been commented upon previously in the discussion of this disease in rabbits.[5] This impression is supported also by the absence of any new abscess formation in kidneys with healing lesions. Even in those animals with active pyelitis and positive cultures for 30 weeks, no evidence of recent invasion of the parenchyma was seen. Further support for this comes from the fact that kidneys examined six months and one year after inoculation of bacteria did not seem in general to be more severely scarred than those examined just a few weeks after injection. It would appear then that the model of pyelonephritis under study does not produce a lesion which is progressive; damage of the renal parenchyma did not increase with the passage of time.

Correlation of morphological and bacteriological data shows that disappearance of viable bacteria from a previously infected kidney usually takes at least six weeks, since only after this time was definite morphological evidence of previous infection detected in a sterile kidney. Active pyelitis began to be seen six weeks after injection of organisms. At 30 weeks this renal lesion was closely associated with viable bacteria in the kidney. In two kidneys with severe active pyelitis, no other renal lesions were detected.

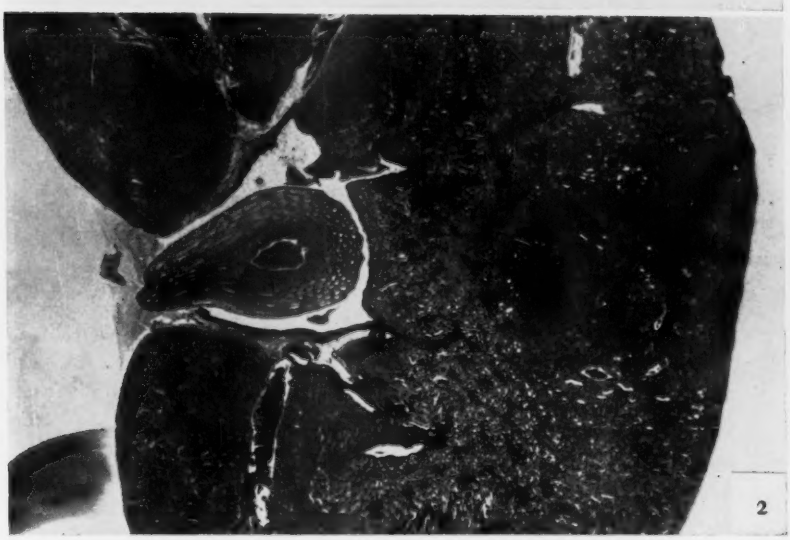

FIG. 1. Gross appearance of kidneys one week after the intravenous inoculation of $10^8$ staphylococci showing wedges of inflammation extending from medulla to cortex.

FIG. 2. Low power view of kidney examined at the same interval as kidneys shown in Figure 1. Abscess involving over one-half the diameter of the papilla in this section. Cortical inflammation limited to abscesses shown.

3

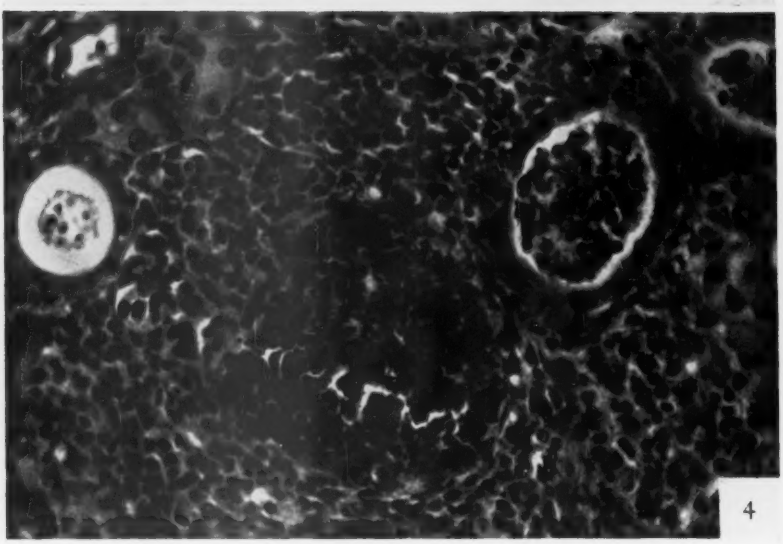

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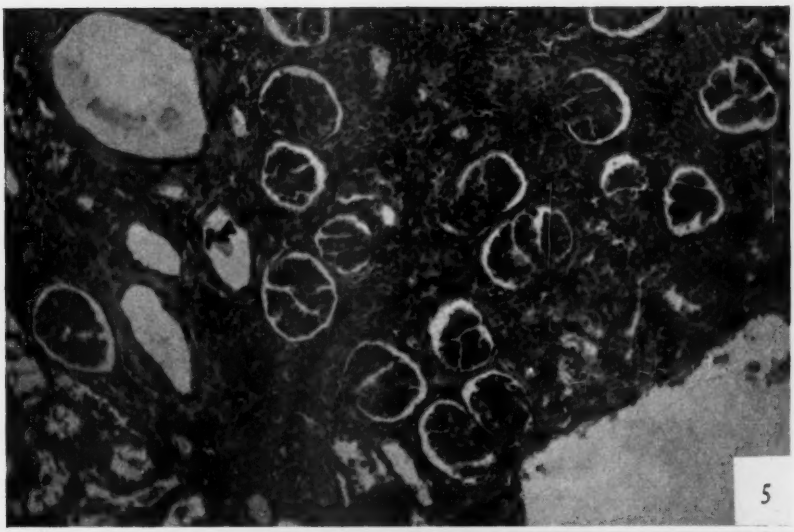
FIG. 3. The necrotic tip of the papilla has fallen out of both halves of this kidney examined one week after the inoculation of organisms.

FIG. 4. Microabscess one week after inoculation of bacteria. Polymorphonuclear leucocytes are visible in tubular lumina.



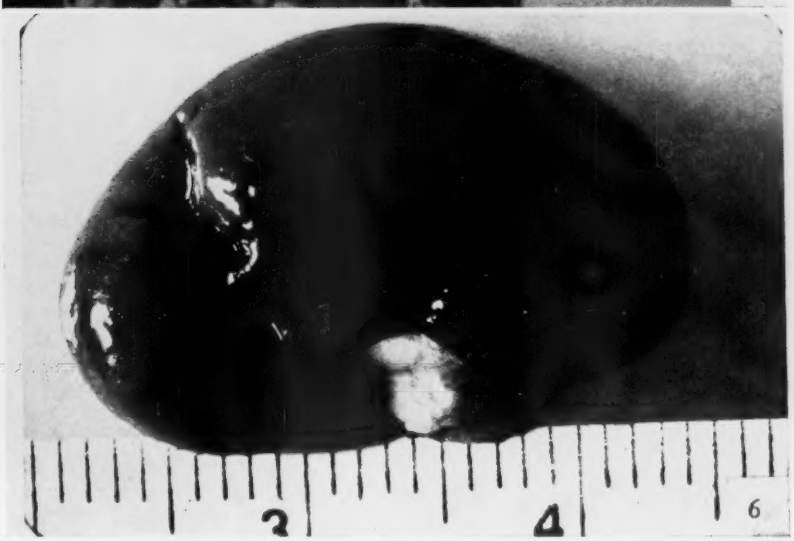
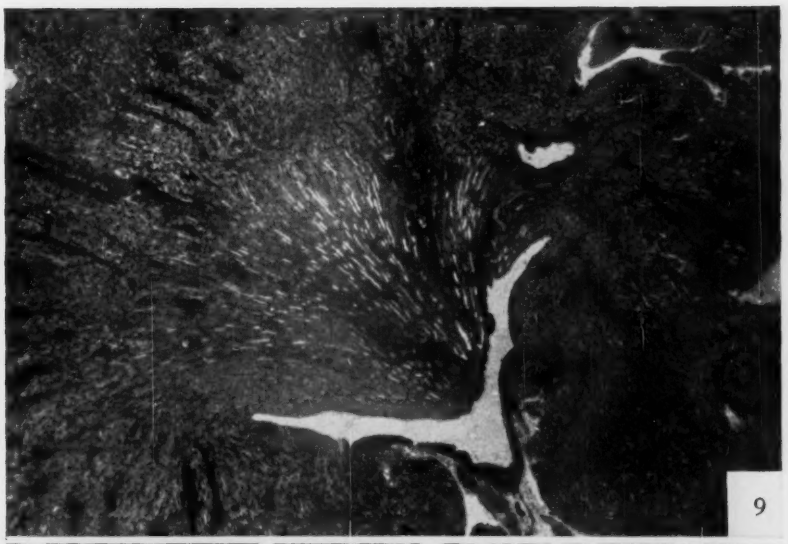




FIG. 5. Typical cortical scar four months after infection. The glomerular capillary tuffs are normal.

FIG. 6. Gross appearance of cortical scars five months after infection.

FIG. 7. Medullary cyst formation underlying cortical scar. Papillary scarring is not visible in this section.

FIG. 8. A dense, dark layer of polymorphonuclear leucocytes is seen lining the renal pelvis. No other lesions were found in this kidney either on gross or microscopic examination.



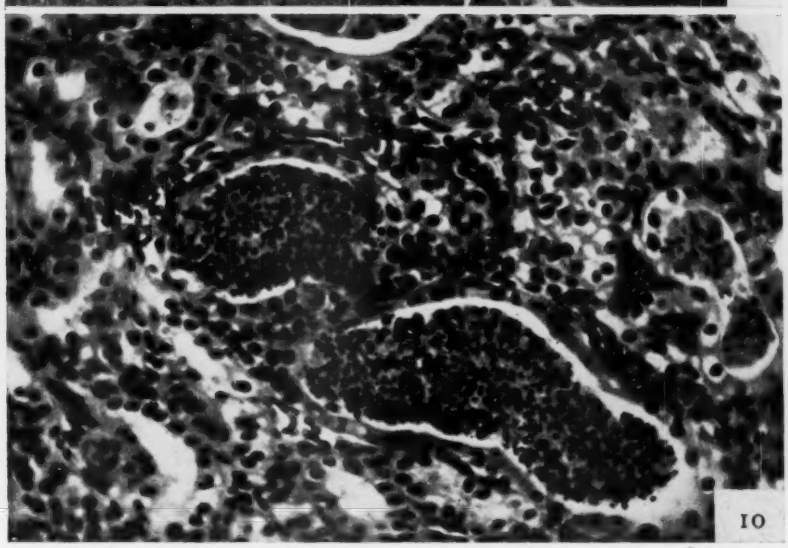
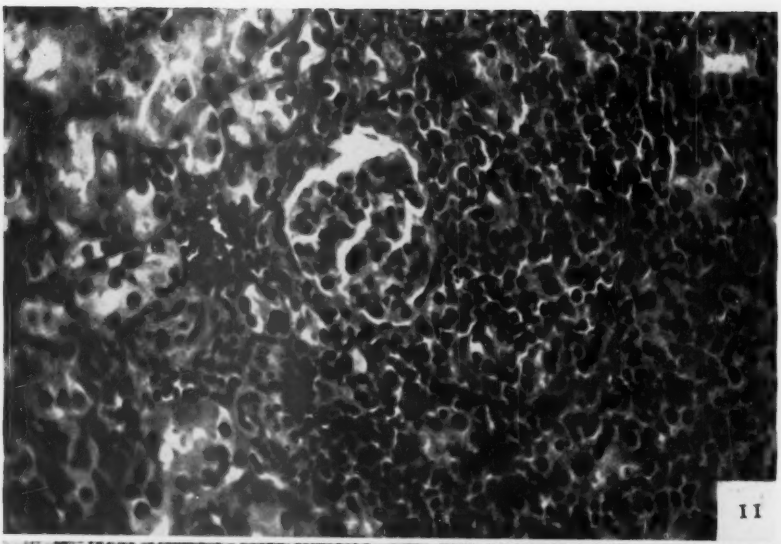




FIG. 9. Darker zones in papilla indicate scar remaining five months after infection.

FIG. 10. Dilated tubules filled with polymorphonuclear leucocytes in a kidney whose ureter was ligated for three weeks. No infection. Compare with Figure 4.



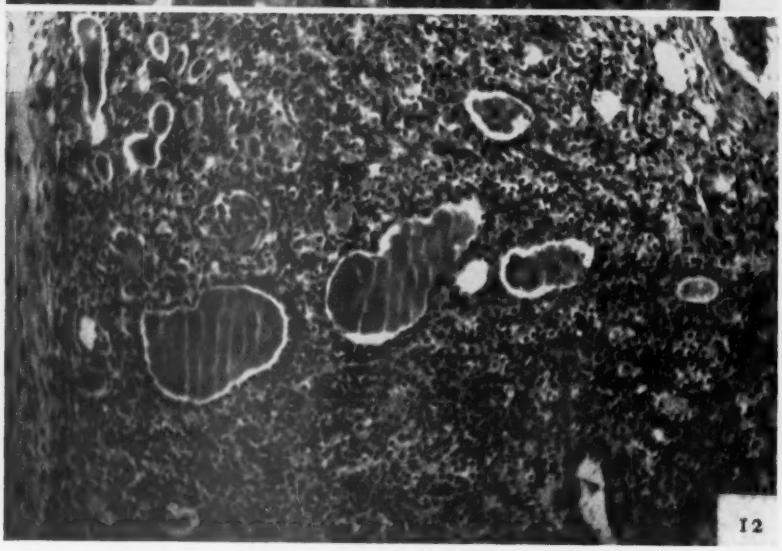
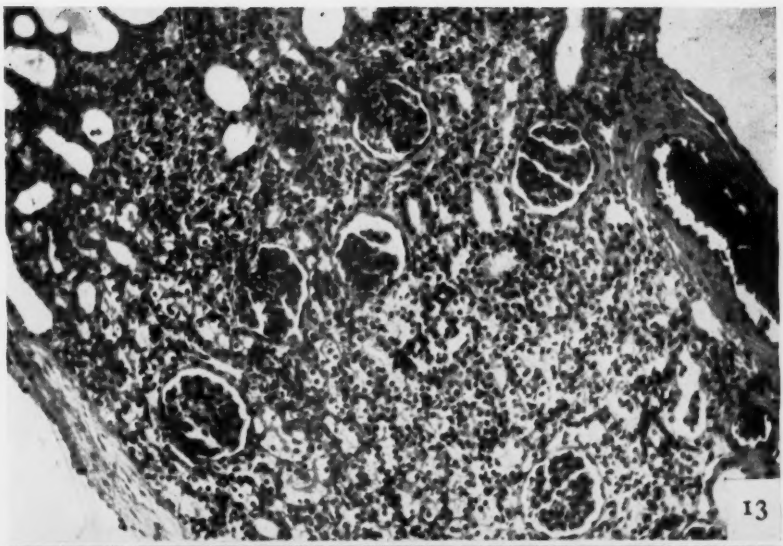
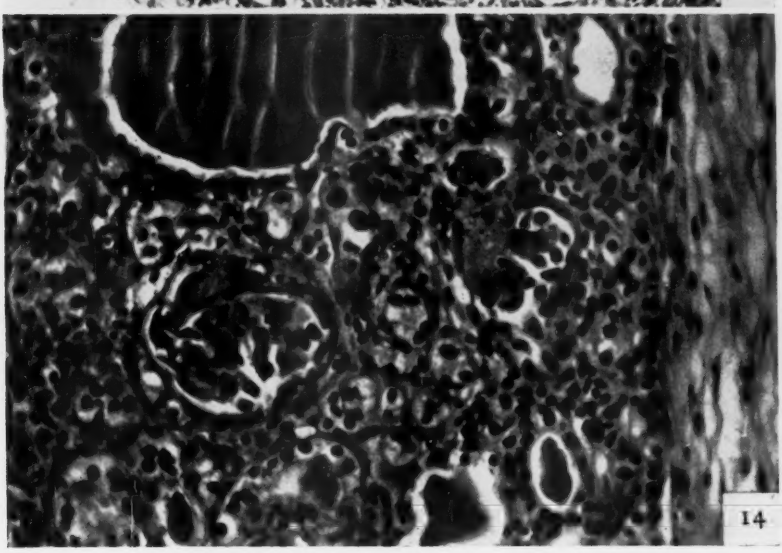




FIG. 11. Cellular exudate composed of polymorphonuclear leucocytes, lymphocytes and plasma cells, three weeks after ureteral ligation without infection.

FIG. 12. Colloid cast formation three weeks after ureteral ligation without infection.




FIGS. 13, 14, 15. Glomerular crowding and hyalinization three weeks after ureteral ligation without infection.

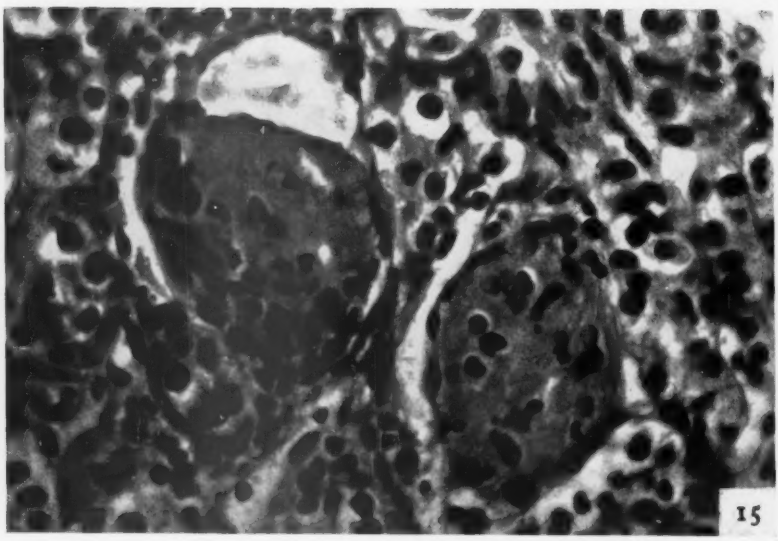



FIG. 15. See legend Figs. 13, 14.

It is apparent that pyelitis is a common finding in hematogenous staphylococcal pyelonephritis in rats. Occasionally it may be the only detectable morphological abnormality of the kidney.

The morphological findings in the present study do not differ significantly from previous descriptions of staphylococcal pyelonephritis in the mouse and rabbit. However, since ureteral obstruction was not a necessary first step in the production of this infection, it is pertinent to examine the differences that exist between the present disease and the one produced with *E. coli* following ureteral ligation. The studies using *E. coli* were carried out in the rabbit, a fact which in itself may explain some of the differences. Nevertheless, it is important to try to distinguish the changes due to obstructon and infection from those due to infection alone.

The presence of colloid casts in the kidney is considered to be one of the most characteristic histological features of chronic pyelonephritis. Mallory, Crane, and Edwards have stated: "If our interpretation of the formation of these colloid casts is correct as given, their presence should be the most reliable criterion for the diagnosis of the healed lesion (of pyelonephritis)... Acute pyelonephritis is probably the only lesion of the kidney which could produce such a combination of circumstances."[11] These observations have been confirmed by Heptinstall and Gorrill, in experiments which were also carried out with *E. coli* using ureteral obstruction.[14] It was striking, therefore, that such casts were only rarely seen in rats with staphylococcal renal infection in any stage of disease up to one year after infection. It is of interest that Heptinstall and Gorrill also observed infrequent cast formation in rabbits given staphylococci without ureteral ligation.[14] Most authors have agreed that colloid cast formation was dependent upon obstruction of nephrons in addition to infection. Cast formation *was* observed, in the present study, however, in rats whose ureters had been ligated for three weeks in which there was *no evidence of infection.* It would appear that the formation of colloid casts in dilated nephrons is dependent primarily upon obstruction, not infection.

The distention of tubules with leucocytic casts was also seen in sterile obstruction along with crowding of glomeruli, tubular atrophy, and marked lymphocytic interstitial infiltration. Helmholtz and Field have commented on this.[13] This tissue reaction is no doubt a result of increased tissue pressure and anoxia, both of which are well known to accompany obstruction.[18]

Glomerular hyalinization is another of the changes which has been described in infected obstructed kidneys. Although these lesions were not seen in rats with staphylococcal pyelonephritis, it might be argued that they were missed, or that species difference accounted for a transformation of

glomeruli which made them difficult to identify. These glomerular changes were seen, however, in obstructed kidneys which were sterile. Thus another lesion of pyelonephritis may result from obstruction without infection.

It is apparent that many of the histological features of infection combined with obstruction may be duplicated by obstruction alone. Sometimes, however, infection itself is the cause of obstruction. Furthermore, since many effects of obstruction are related to changes in blood flow and tissue pressure, it is possible that primary vascular abnormalities might also produce tissue changes indistinguishable from those of pyelonephritis.

## SUMMARY

The present report describes the bacteriological course and morphological consequences of pyelonephritis following the intravenous injection of large numbers of virulent staphylococci into normal rats. There is only slight mortality accompanying this infection, the natural tendency of which is to heal in 15-22 weeks. Bacteria were not recovered from the urine until their multiplication was demonstrable in the kidney after which time there was good correlation between the presence of bacteria in the urine and kidney.

Active pyelitis was frequently seen in kidneys six weeks or more following the intravenous inoculation of staphylococci. At 30 weeks this lesion was usually found when viable bacteria were recovered from the kidney. Occasionally, pyelitis was the only lesion seen microscopically. It is evident that inflammation of the renal pelvis is not useful in determining the route by which bacteria reach the kidney.

Histological features considered typical of experimental pyelonephritis, such as colloid casts, polymorphonuclear leucocytic casts, and glomerular hyalinization, were reproduced in sterile obstructed kidneys. Colloid casts were rarely seen and hyalinized glomeruli not detected in the healing stages of staphylococcal pyelonephritis, an infection which does not require obstruction for its initiation. It is suggested that many of the histological changes thought to be typical of renal infection may be produced by obstruction of nephrons.

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# EFFECT OF EXPERIMENTAL PYELONEPHRITIS ON THE RENAL CONCENTRATING ABILITY OF THE RAT§

The intravenous injection of virulent staphylococci produces renal infection in 75 to 100 per cent of normal rats.[1] The renal lesion persists for many months and carries with it little mortality. This model is suitable for the study of disturbances of renal function due to infection, since prior ureteral obstruction or localized renal damage is not required.

The present experiments were undertaken to determine the effect of experimental pyelonephritis upon renal concentrating ability and to correlate this function with the histological and bacteriological findings.

## METHODS

*Group I. Long-term study*

White male Sprague-Dawley rats weighing 160 to 260 grams were allowed free access to Purina Chow and water except during the 24-hour period of food and water deprivation associated with each test of maximum concentrating ability. Each of 20 rats was inoculated with 0.5 ml. of an overnight culture of a hemolytic *Staphylococcus aureus* (Giorgio strain). The inoculum contained $10^8$ bacteria and was injected into the tail vein. Seven rats served as controls.

Maximum urinary concentration ($U_{max}$) and blood urea nitrogen (B.U.N.) were determined on five separate occasions at approximately monthly intervals. The first

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test was performed just prior to the inoculation of the organisms. Seven control rats did not receive bacteria and were tested at similar intervals. After 32 weeks the animals were sacrificed.

*Group II. Short-term study*

Eleven rats were inoculated with 4 x $10^7$ bacteria after an initial test of maximum concentrating ability and blood urea nitrogen. Along with six control rats they were then tested twice more at approximately bi-weekly intervals. In this study, the food intake of each of the control animals was limited to the average amount ingested daily by the inoculated rats. The experiment was terminated after 51 days.

*Bacteriological and pathological studies*

At the time of sacrifice, using aseptic precautions, the kidneys were hemisected longitudinally and inspected. Half of each kidney was homogenized and serially diluted. Agar pour plates were prepared and incubated at 37° C. for 24-48 hours. The remaining half kidney was placed in formalin for histological study. Bladder urine was aspirated aseptically and a loopful streaked on blood agar plates.

*Criteria of infection*

Kidneys which were found to contain more than 100 colonies of staphylococci in either half kidney or which elaborated infected urine were arbitrarily considered to be "infected." Those having fewer than 100 colonies in either half kidney in addition to a sterile urine were designated "non-infected."

The rats designated as "infected" were subdivided on the basis of quantitative bacterial count. Those infected animals with more than $10^4$ viable units in either half kidney in addition to having a positive urine were further designated as "active." Those with $10^3$ colonies and a negative urine, or with less than $10^3$ colonies and a positive urine, were termed "healing."

*Test procedure*

Prior to a given test, food and water were withheld for 24 hours. After the first nine hours, 50 milli-units of vasopressin in oil* were injected subcutaneously. The rats were then placed in metabolism cages, and urine was collected for 15 hours in funnels filled with mineral oil. One ml. of blood for B.U.N. was obtained from the rat's tail. The volume of urine excreted in 15 hours was recorded. Osmolality of the urine was determined with a Fiske osmometer. B.U.N. was measured by the microdiffusion method of Conway.[2]

## *Group I. Long-term study (Table 1)*

Of the 20 rats originally inoculated, 13 were infected. Of the 13 infected rats, 6 had active infection and 7 had a healing lesion. The $U_{max}$, BUN and total solute output ($U_{osm}V$) for each monthly test are shown in Table 1.

In the infected group there was a marked drop in maximum urinary concentration in the first study following the inoculation of bacteria (Table 1,

TABLE 1. GROUP I. PYELONEPHRITIS. LONG-TERM STUDY. 32 WEEKS.

| *Test number* | *1* (0 Weeks) | *2* (4 Weeks) | *3* (12 Weeks) | *4* (19 Weeks) | *5* (26 Weeks) |
|---|---|---|---|---|---|
| Non-infected (7 rats) | | | | | |
| $U_{max}$ mosm Kg. | 2272 ± 521* | 1391 ± 332 | 1767 ± 423 | 1910 ± 237 | 2007 ± 221 |
| p** | — | < 0.01 | — | — | n.s. |
| BUN mgms/100 ml. | 17.0 ± 2.1 | 21.5 ± 5.2 | 21.2 ± 3.3 | 19.7 ± 4.6 | 19.1 ± 4.0 |
| p | — | n.s. | — | — | n.s. |
| $U_{osm}V$ mosm/15 hrs/100 gm. | 3.21 ± 0.97 | 2.61 ± 0.58 | 2.20 ± 0.47 | 2.04 ± 0.46 | 2.01 ± 0.43 |
| p | — | n.s. | — | — | n.s. |
| Infected healing (7 rats) | | | | | |
| $U_{max}$ mosm Kg. | 2480 ± 334 | 1534 ± 363 | 1954 ± 445 | 2060 ± 170 | 2345 ± 147 |
| p | — | < 0.005 | — | — | n.s. |
| BUN mgms/100 ml. | 19.5 ± 4.8 | 19.8 ± 4.0 | 21.3 ± 1.8 | 19.7 ± 2.0 | 19.2 ± 1.6 |
| p | — | n.s. | — | — | n.s. |
| $U_{osm}V$ mosm/15 hrs/100 gm. | 2.95 ± 0.82 | 2.02 ± 0.67 | 2.18 ± 0.31 | 1.75 ± 0.28 | 1.41 ± 0.42 |
| p | — | < 0.05 | — | — | < 0.005 |
| Active infection (6 rats) | | | | | |
| $U_{max}$ mosm/Kg. | 2517 ± 237 | 1557 ± 216 | 1619 ± 339 | 1749 ± 356 | 1841 ± 359 |
| p | — | < 0.001 | — | — | < 0.01 |
| BUN mgms/100 ml. | 16.6± 1.9 | 21.5 ± 6.0 | 22.3 ± 3.1 | 20.5 ± 2.6 | 19.4 ± 2.3 |
| p | — | n.s. | — | — | n.s. |
| $U_{osm}V$ mosm/15 hrs/100 gm. | 2.82 ± 0.51 | 2.26 ± 0.49 | 2.21 ± 0.39 | 1.99 ± 0.62 | 1.93 ± 0.29 |
| p | — | n.s. | — | — | < 0.025 |
| Control (7 rats) | | | | | |
| $U_{max}$ mosm/Kg. | 1809 ± 340 | 2471 ± 222 | 2418 ± 279 | 2437 ± 300 | 2425 ± 277 |
| p | — | < 0.005 | — | — | < 0.005 |
| BUN mgms/100 ml. | 21.7 ± 3.9 | 19.6 ± 2.9 | 18.5 ± 1.2 | 20.0 ± 2.2 | 20.0 ± 2.0 |
| p | — | n.s. | — | — | n.s. |
| $U_{osm}V$ mosm/15 hrs/100 gm. | 2.54 ± 0.56 | 1.94 ± 0.39 | 1.35 ± 0.29 | 1.51 ± 0.30 | 1.60 ± 0.16 |
| p | — | < 0.05 | — | — | < 0.005 |

* Mean ± standard deviation. ** p value is for each test compared with test 1.

test 2). In the animals with active infection, concentrating ability remained impaired, whereas the $U_{max}$ of the "healing" group, despite a similar initial drop, gradually returned towards its original value. The BUN was not significantly altered during the period of observation, and the total solute output was not increased. The control rats had an initial $U_{max}$ which was low, possibly because these animals were smaller than the experimental rats

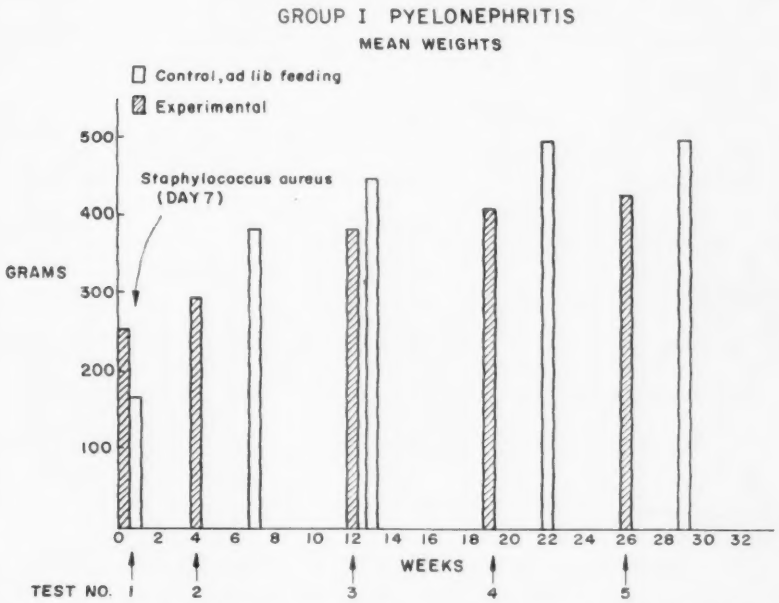


FIG. 1. Mean weight of the experimental and control rats of group I long-term study.

(160 grams as compared with 260). Subsequently, however, these rats maintained a $U_{max}$ in the expected normal range. The weights for the 7 control and 13 infected rats are shown in Figure 1.

The "control" animals were not pair-fed and were smaller than the experimental animals. The data from these controls serve then only to demonstrate that ability to concentrate urine does not decrease with advancing age on the given diet. The critical studies here are those comparing the infected and non-infected members of the inoculated group.

* Pitressin Tannate in oil was kindly supplied by the Parke-Davis Company.

*Group II. Short-term study (Table 2).*

Of the 11 test rats, 5 were infected at the time of sacrifice. The infected rats developed a significant decrease in $U_{max}$ which was even further decreased on the third test, 44 days after injection of bacteria. The pair-fed

TABLE 2. GROUP II. PYELONEPHRITIS. SHORT-TERM STUDY. 51 DAYS.

| *Test number* | *1* (0 Days) | *2* (23 Days) | *3* (44 Days) |
|---|---|---|---|
| Non-infected (6 rats) | | | |
| $U_{max}$ mosm/Kg. | 2319 ± 308* | 2367 ± 343 | 2416 ± 112 |
| p** | — | n.s. | n.s. |
| BUN mgms/100 ml. | 24.2 ± 3.9 | 22.8 ± 4.4 | 20.7 ± 2.4 |
| p | — | n.s. | n.s. |
| $U_{osm}V$ mosm/15 hrs/100 gm. | 2.26 ± 0.56 | 2.99 ± 1.29 | 2.48 ± 0.97 |
| p | — | n.s. | n.s. |
| Infected (5 rats) | | | |
| $U_{max}$ mosm/Kg. | 2750 ± 217 | 1889 ± 402 | 1597 ± 283 |
| p | — | < 0.005 | < 0.001 |
| BUN mgms/100 ml. | 25.2 ± 2.8 | 25.0 ± 3.1 | 19.8 ± 2.5 |
| p | — | n.s. | < 0.005 |
| $U_{osm}$ Vmosm/15 hrs/100 gm. | 1.87 ± 0.46 | 2.83 ± 0.69 | 2.50 ± 0.09 |
| p | — | < 0.05 | < 0.05 |
| Control pair-fed (6 rats) | | | |
| $U_{max}$ mosm/Kg. | 2727 ± 189 | 2073 ± 451 | 2538 ± 262 |
| p | — | < 0.01 | < 0.05 |
| BUN mgms/100 ml. | 21.3 ± 3.5 | 19.4 ± 2.1 | 17.9 ± 2.8 |
| p | — | n.s. | n.s. |
| $U_{osm}V$ mosm/15 hrs/100 gm. | 2.14 ± 0.31 | 2.2 ± 0.29 | 1.83 ± 0.11 |
| p | — | n.s. | < 0.05 |

* Mean ± standard deviation.
** p value is for each test compared with test 1.

controls, restricted in their food intake, developed a less marked but significant drop in $U_{max}$ at the time of the second test which returned towards the original value two weeks later in the third test. Unlike the infected animals, however, the $U_{max}$ of the non-infected rats was unchanged. Those non-infected rats which ate well consistently weighed more than the infected rats and had no change in concentrating ability. Solute excretion

increased slightly, and the BUN was unchanged except in the last examination of the infected group where the BUN was noted to be lower. The weight curves for this group are shown in Figure 2.

## DISCUSSION

Staphylococcal pyelonephritis in the rat is associated with a marked decrease in maximum urinary concentrating ability. The initial impairment of concentrating ability can be partially attributed to a decrease in food

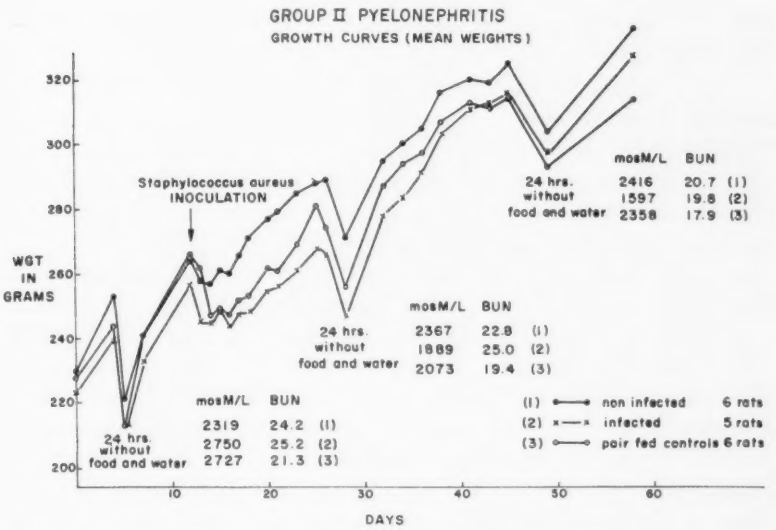


FIG. 2. Mean weight of the experimental and control rats of group II short-term study.

intake as well as to the presence of active infection in the kidney. With recovery from the acute effect of bacteremia and the restoration of normal food consumption, as reflected by the gain in weight shown in Figure 1, the factor of inadequate protein intake, known to influence concentrating ability,[8] no longer can account for the observed changes in $U_{max}$. Renal function, as judged by maximum concentrating ability, then depends on the subsequent course of the infection; healing is associated with a return of function, whereas persistent active infection, in spite of adequate food intake, continues to impair concentrating ability.

The changes in concentrating ability can not be attributed to changes in blood urea nitrogen or solute output in group I. The increased excretion of solute noted in the infected rats of group II did not exceed the value observed in the non-infected rats. These facts suggest that the decrease in $U_{max}$ was not due to a change in glomerular filtration rate or to a solute diuresis.

Similar observations have been made in patients with urinary tract infection. In children, Winberg[4] found concentrating ability to be impaired while creatinine clearance remained normal. After appropriate antimicrobial therapy his patients improved their ability to concentrate urine. Kaitz[5] also reported diminished concentrating ability in patients with asymptomatic bacteriuria in the presence of a normal creatinine clearance.

The low BUN in the infected rats of the short-term study might be interpreted as indicating an increase in renal blood flow and glomerular filtration rate, a response which has been observed in man during acute renal infection[6] and in response to pyrogens.[7] The correlation of renal concentrating ability with the stage of infection suggests that impairment of function is related to some consequence of the presence of bacteria within the kidney. Much of the destruction of renal tissue which is apparent on histological examination of kidneys during the acute stage of infection appears to be irreparable.[1] Since the disturbance in renal concentrating ability is at least partially reversible, it would seem that disturbances of blood flow or impairment of the function of neighboring medullary tubular cells was responsible for the temporary decrease in concentrating capacity observed in rats of the present experiments. Proportional to its size, a given area of inflammation in the tiny pointed papilla of the rat kidney is likely to disturb renal function to a much greater degree than if it were located in the larger cortical zone of the kidney. A function of the renal medulla, such as concentrating ability, might therefore be expected to undergo disproportionate and reversible impairment when inflammation is located in the medullary zone.

It is conceivable that recovery of function has little to do with subsidence of inflammation but rather with compensatory hypertrophy of remaining nephrons. If this were the case, then failure of recovery in a kidney with persistent infection would suggest interference with this hypertrophy by infection. Such interference would have important implications but for the moment remains unproved. Clearly, any study of the effect of pyelonephritis on renal function must include consideration of the status of bacterial infection within the kidney.

SUMMARY

1. Pyelonephritis was produced in rats by the intravenous injection of a virulent strain of *Staphylococcus aureus*.

2. There is a marked decrease in maximum concentrating ability which, in the presence of active infection, persists beyond the initial period of inadequate food intake.

3. The decrease in concentrating ability does not seem to be due to an alteration in glomerular filtration or solute output.

4. Restoration of concentrating ability accompanies the healing process.

5. It is suggested that the observed impairment of concentrating ability was due in part to the consequences of renal inflammation rather than solely to destruction of nephrons.

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## POPULATION PROBLEMS IN A CONTRACTING WORLD**

The population problem has been a popular theme for a decade or more. About every possible medium from scientific and literary journals to the daily press and television, has had a hand in presenting its multiple details and controversies. The result is a familiarity so universal that a summary satisfies present purposes. The main objective is to state the particular obligations of medicine and public health in a difficulty that engages all of society, and involves most of its interests and agencies.

The present rapid growth of population, the responsible factors, and the resulting imbalance between people and resources create a critical social and economic problem of today. It has its health component[1] as do most such difficulties; in this instance especially so, because medical progress is frequently responsible for the situation of too many people. In many countries birth rates continue high while death rates decline; the demographic gap widens and the additions to numbers accelerate.

Overpopulation too often is resolved into a question of food supply, the food chain concept of ecologists. The precept is that a species cannot permanently exceed its food supplies, that these fix an upper limit of numbers. This is an oversimplification. Man has other important values such as the need for clothing, shelter, and adequate medical facilities; the desire for education, employment and recreation; the demand for cultural and spiritual growth as well as physical well-being. By such criteria is the existence of a population problem determined defining an unsatisfactory balance between population and all resources.

Numbers of people do not of themselves produce a population problem. The important consideration is the relation between the two variables of people and resources. India, a country with recognized difficulties has 402 million people in an area of 1,221,880 square miles. Greenland in 1957 had a population of 28,206 with an area two-thirds that of India, and yet its population problem was of relatively greater moment. Because of climate

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and topography, the demands on food and other resources were beyond local supplies or the local economy. The natural increase in numbers in Greenland was 3.6% per year, much in excess of India's 1.6%.

## THE WORLD AND ITS PEOPLE

The present population problem is of relatively recent evolution, considering the history of mankind. Three hundred years ago the people of the world numbered about half a billion; there had been no outstanding increase for thousands of years. Within about two centuries, that figure doubled, but only 80 years were necessary before it doubled again, to two billion in 1930. Since then, within a period of 30 years, the world population has grown to 2.6 billion.

Populations of the world once were grouped into three general classes. The first included countries of Africa and the Far East, with high birth rates and high death rates. The second was recognized as in a transitional stage, with persisting high birth rates and decreasing death rates, countries represented by India, Ceylon and many in South America. In a third category were nations of the Western world, marked by low birth rates and low death rates. The distinction is now not nearly as clear as before World War II. Recent years have seen broad changes. The average population increase for the world as a whole was 1.1% per year in 1947. It is now estimated at 1.7%, greater by close to a third within less than 15 years.

The best estimates[2] for the future United States population are 239 millions in 1980, essentially a 30% increase from the present 180 millions. In 1980 the world population is likely to be 4 billion. Rising numbers are understandable. What is not so regularly appreciated is that even with an immediate and decided decrease in general birth rates, and that is unlikely, the forces now in motion would continue to provide a population problem in many parts of the world for many years. The need for action is evident. From a world view-point the population problem is more than a threat of the future, a concern of something to be avoided; it is here now.

Population problems know neither time nor boundaries.[3] During the thousands of years that world population increase was 0.2% per year, the problem was survival. A common misconception is to relate the present difficulties of overpopulation to far away places and countries in transition to an industrial economy. Despite an unusually favorable position in natural resources and material economy, the United States has its population problems. The main question is whether the expected gains of the future, distributed among greater numbers of people, will suffice to maintain the rising standard of living so characteristically American. The troubles in

the suburbs, the crowded highways, and the shortage of schools are minor distractions of the moment.

Population growth in other countries of the Americas is rapid; in Mexico, for example, at 3% per year, it exceeds all large countries of the world. Central America and the Caribbean area as well as South America follow closely. If present conditions continue Latin America will soon exceed English-speaking North America in numbers, with a growing industrial potential not to be discounted. Asia has always had half of the world's people; it is more than that now, and a growing nationalism and a developing industrial economy make for another sort of Asia. A goodly part of the population problems of the United States is international, bearing on our national security, commerce, and industry and influencing the obligations we have as one of the dominant powers of the world. This is the reality of the contracting world of today.

## PRINCIPLES OF POPULATION GROWTH

Population change is a function of births, deaths, and migrations. Medical progress has led to a remarkable decline in death rates, sometimes spotty, commonly uneven, but more or less universal. Birth rates have tended to be more fixed. The demographic gap, the difference between birth rates and death rates, is the index of natural increase in numbers, currently about 1.7% for the world as a whole, exceeding 4% in some few regions, and in no major country stabilized at parity or marking a decline.[4]

Increased life expectancy is a second factor promoting greater numbers. As one demographer[5] compares the present American situation with the past, half as many children are born and they live two and a half times as long. The gain is in life expectancy at birth; not much change has occured in length of life after reaching age 65 years. The highest values are in the Western countries, but other nations are recording gains within years that took Europe decades and sometimes centuries to accomplish.

Rates of population growth commonly differ by age and sex,[2] largely determined by demographic events of the past. The expectancy in the United States for children and adolescents by 1980 is measurably less than the average increase of 30%; for ages 15-44 it is more, about 40%. The age group 45-64 will likely show an increase of 20%, again less than average. The age 65 and more promise the greatest increase; for males about 40%, and for females almost 60%, twice that for the general population. The existing predominance of older women over men presumably will be greater than ever, the ratio changing from about 7:6 to 7:5.

Geographic shifts in the United States population are no less than remarkable. Migrations are of consequence in the population dynamics of individual localities or regions within a country; currently they are of lesser importance internationally. Urbanization and suburbanization are well recognized. The possible extent of such changes is illustrated by the Negro population of this country. In 1900, 20% of colored people lived in cities; in 1960 the estimate is 70%. Changes in density and geographical concentrations of people have an impact on birth and death rates, through altered environment, occupation, differences in manner of living and, incidentally, in facilities for medical care.

## MEDICINE'S PART IN POPULATION DYNAMICS

An evaluation of population numbers is primarily a function of demography, but requiring the skills of a variety of medical, biological, and behavioral sciences. The decision as to need of a program for control, and plans for its administration, bring into play numerous agencies of society, governmental and otherwise. This is so because the population problem touches on almost all aspects of human welfare. The health professions have no more obligation than anyone else and yet some of the necessary action and research is peculiarly within the competence of medical practitioners, administrators, and investigators. Professional self-interest as well as duty compels a familiarity with demographic principles and methods. Perhaps it is well to start with self-interest, in viewing what is involved.

*Planning for Medical Care.* Any reasoned plan for future needs in medical care takes into account demographic projections of the population to be served,[8] their numbers, the kinds of people by age, sex and other characteristics, and distributions by time and place. As has been demonstrated, populations grow unevenly according to these variables and the particular groups have different kinds and incidence of disease.

Medical and other administrators have to plan for future facilities in management of patients in order to include numbers and kinds of hospital beds, clinics, laboratories, provision for rehabilitation, nursing homes, pharmacies, and convalescent centers. Official and voluntary health agencies, including those of industry, must adapt service arrangements to the changing character of a community. The medical educator has decisions to make on numbers and qualifications of students, the facilities or education and training, programs of instructions as well as teachers. A knowledge of general demographic trends and of the local or specific situation makes possible exact appraisal of requirements.

*Administrative Program for Population Control.* India, Pakistan, and Japan, the three countries with organized programs for population control, have placed the responsibility with the national organizations for public health. The American Public Health Association[7] includes a concern with population dynamics among its established policies, and official and voluntary health agencies have recognized a responsibility at the local and state level.[8]

The health department would appear to be the logical agency of society for the practical work of birth control, having a trained staff accustomed to contacting people in their homes, technical methods applicable to family planning, and an extensive experience in education of the public. There is a more fundamental justification for that choice.

Any physician would prescribe as principle an equal attention to births and deaths in a program for population control. There is no advantage in saying that the central difficulty is with natality, but improved control of deaths cannot be neglected because of the exigencies for control of births. The two are part of a single problem.

Deaths have declined by reason of medical progress implemented in its practical applications by government and society; the individual has had secondary responsibility. The control of births presents the opposite situation and is the harder task. Motivation is a practical consideration. The demonstration that five children are no longer necessary to assure that three live to become adults is a strong incentive. More survivors provide visual evidence of the difficulties in providing proper education and material advantages. These are some of the reasons why Frederick Osborn[9] has stressed decreased death rates as the first step in population control.

The family has been characterized as the unit of overpopulation. Society can contribute to the control of births to an appreciable degree, but the main obligation falls on the individual. This sets a direct responsibility on practicing physicians to encourage motivation and to assure maximum accomplishment by advice on method. The physician is in the prime position to do this as family counsellor in health matters, and the mechanism is preventive medicine as opposed to public health.

These practical relationships are part of the reason that the principles of demography are finding a place in present-day instruction in preventive medicine.[10] The more fundamental incentive is a growing appreciation by the health professions of an obligation to contribute within their capabilities to the broad social problem involved in population dynamics, not alone through public health and clinical activities, but particularly through research in those features of demography relating to medical matters.

*Evaluation of Medical Progress.* In countries with high death rates, considered on a short-term basis, numbers of deaths are a sensitive variable in population growth and judge the improvement in public health programs. A sharp advance in methods for treatment or prevention of a disease can alter most demographic calculations, as happened when the antibiotics and modern insecticides became available against infections. The steady advance of general therapeutic and preventive measures is less spectacular but the cumulative effect is ultimately significant. A continuing medical progress is certain: reliable estimates of the effects on future population growth require the technical competence of the physician.

*Clinical and Field Trials of Contraceptive Measures.* Clinical trials under the controlled conditions of hospital or outpatient department and subsequent field trials in general population are medical affairs. Few contraceptives have been tested by field trials that meet the principles prescribed by Bradford Hill[11] or the practical procedures outlined by Witt.[12] In their full potentiality they lead directly to the more fundamental endeavor of a search for new facts.

*Physiology of Human Reproduction.* Better understanding of the processes of fertility is urgently needed. Birth rates are the resultant of a variety of biologic, social, and psychological influences. The answer to the main biologic questions is through laboratory research, by its nature a function of the medical and allied sciences. The discovery of a simpler and more effective contraceptive would undoubtedly facilitate acceptance and practice of birth control. It is not the critical consideration so frequently presumed. Some one of the existing contraceptive procedures is acceptable to any particular culture or people, and sufficiently effective to attain a reasonable result. The difficulty is to get people to use them, which places the emphasis on social and psychological determinants.

*Experimental Ecology of Population Growth and Variation.* The principles of population dynamics are amenable to study by the methods of experimental ecology, using colonies of vertebrates, with the unit of observation a population in contrast to individuals in series. The fundamentals are to be found in the work of Pearl[13] on fruit flies; technical method and procedure are to be had from the experiments of Greenwood *et al.*[14] and of Webster.[15] Manipulation of births, deaths and migrations, as well as food and climate can be studied for their effects on age and sex distributions, and growth or decline of the experimental populations. The mass or population effect of introduction of a biological contraceptive is another attractive

possibility. The studies next to be described are based on these ecologic precepts, differing mainly in that they involve populations of people observed under natural conditions.

*Field Studies in Population Dynamics.* The epidemiological field method in this instance is that of incidence investigation as contrasted to prevalence survey. The procedure has proved useful in the study of diseases of endemic nature, slow evaluation, and long duration, as they affect populations of people; notably in heart disease. Population dynamics have these characteristics. This particular method is time-consuming and expensive but experience has demonstrated its essential value over short-term cross-section surveys.

The principle is an intensive inquiry, prospective in type, concerned with small populations representative of the larger area and extending over sufficient time to acquire significance. The present study began in the Punjab State of India in 1953, with a population of some 16,000 persons and observations continued until March 1960. It was built around the introduction of a contraceptive measure, the foam tablet, in a group of seven rural villages with nine others as controls.

The method has many advantages. The investigators live and work in the community. The aim is to seek out events rather than wait for them to be reported, either to the investigator or officially. The study deals with incidents as they occur. The data attain an accuracy not possible in information gathered by statement of the person concerned on what has happened in the past.

Much of what is known of populations relates to an observed or determined status at a point in time. Changes are identified by serial comparisons. A main deficiency is the limited and often indirect knowledge of the dynamics of the process, the forces responsible for observed differences. A full understanding requires a search for causes, the many faceted causality as it involves biological, social, behavioral, and economic factors such as the causes related to births, deaths, and migrations.

This intensive field method of investigation is offered in enlargement of the facts to be had by the more conventional methods of demographic analysis[17] concerned with populations of a whole region or country, where the major sources of data are records of the official census of populations, registered births and deaths collected by public agencies, and other information gathered from social and economic facts. In pre-industrial countries vital statistics commonly lack reliability, sometimes to such extent that only the crudest estimates are possible. As a consequence, field studies such

as the Indian National Sample Surveys are common practice attempting to compensate for these recognized shortcomings.

The need for kinds of information not available from existing sources has led demographers to institute field investigations[18] usually in the form of surveys by interview technics, commonly concerned with point prevalence, and occasionally with periodic prevalence through repeat interviews at intervals. The necessity for field study is generally accepted. There is a limit to the usefulness of surveys. The specific need is believed to be a better understanding of the natural history of populations through systematic, continuing observation.

The incidence study presumbly has its greatest value in pre-industrial populations, and especially those of rural areas. The procedure is familiar to public health workers. The value of intensive study of small numbers is long established in medicine; it is to be remembered that Richard Bright described nephritis by observation of 23 patients. A recent colloquium of the French International Union for the Scientific Study of Population[19] endorsed the principle of continuous observation in small areas and in the greatest possible detail as the most direct means under present conditions toward an understanding of African demography.

A main advantage of the method is that specific studies may be designed to measure relationships between aspects of population and a particular environmental, social, or health condition. Two illustrations are cited from the Punjab studies, one in respect to deaths, the other to births. Causes of death were identified by case study for age and sex in addition to the usual determination of numbers. A high morbidity and mortality beyond the first year of life is characteristic of pre-industrial populations, but reported numbers and frequencies are grouped ordinarily within ages 1 to 4 years. By separating individual years, excess deaths were found to center in the second year of life, and the cause was primarily weanling diarrhea. Family structure was analyzed in relation to changing numbers, to birth intervals as influenced by outcome of the preceding pregnancy, and the morbidity and mortality associated with differences in the spacing of children. The aim was to determine optimal intervals and the health costs involved in variations.

The accurate data derived from such studies have the further practical value of providing a corrective factor or index permitting closer estimates of reported birth and death rates. The usual circumstance in countries where reliable data are most needed is that numbers of births and deaths are measurably greater than reported, while causes remain much of a mystery.

## SUMMARY AND CONCLUSIONS

There is more to the population problem than numbers of people and a commensurate food supply. Spiritual and cultural values are not to be submerged in a struggle for existence.

The view is too common that population problems of the present are restricted to pre-industrial countries in underdeveloped regions. Population problems differ in kind and in urgency, but they exist in most countries including the United States.

An increase of population within limits is wholly desirable. The critical question is to define those limits, that is the concentration of people beyond which a nation cannot maintain or gain reasonable standards of health or assure a cultural and economic development.

Each country needs to assess its situation in respect to population dynamics. Decision on the necessity for population control is a country's own individual right and its own direct responsibility. In a contracting world, the weight of that decision extends beyond its borders; the world problem overshadows its parts. Duty as well as self-interest require the better situated societies to lend aid in the search for the essential facts of population growth, in providing technical skills, and in the training of workers in population control.

The population problem relates to many interests and many fields of knowledge. Medicine is one. Its distinctive contributions are through the concept of human ecology and a knowledge and research potential derived from long familiarity with two of the essential elements, births and deaths.

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## Renal Glomerular Lesions in Mice following Injection of Streptococcal M Protein.

By Fred S. Kantor,* Department of Internal Medicine, Yale University School of Medicine.

Type-specificity of Group A streptococci is dependent upon the M antigen. This protein interferes with phagocytosis and is an important determinant of virulence.[1] Recently some preparations of M protein have been shown to precipitate fibrinogen *in vitro*.[2] In an effort to find expression of this new biological property *in vivo*, M protein was injected into mice. The renal glomerular lesions observed form the basis of this brief report.

### MATERIALS AND METHODS

A partially purified preparation of M protein was made by the method of Lancefield and Perlmann[3] from type 1 streptococci, strain No. 2788, kindly supplied as phenolized cells by Dr. C. P. Hagarty of Merck, Sharp and Dohme Laboratories. Electrophoretic examination of this material revealed migration as a single peak, but with sufficient boundary spreading to suggest some heterogeneity. The M protein reacted with absorbed antisera in concentrations as low as 5$\gamma$/ml., and precipitated human and bovine fibrinogens as previously reported.[2] No further purification was attempted.

Human serum albumin (HSA) was obtained through the courtesy of the American Red Cross. Concentration of protein was calculated from the optical density at 280 m$\mu$. in the Beckman spectrophotometer by reference to a protein standard.

### RESULTS

A group of four male Swiss mice averaging 25-30 gm. was given three intravenous injections, within 24 hours, of 5 mg. of M protein dissolved in 0.5 ml. of 0.01 M phosphate buffered saline at pH 7.2. Control mice were similarly injected with human serum albumin. All animals were sacrificed 24 hours after the last injection, and tissues fixed in 10 per cent formalin. Sections of heart, lung, liver, spleen, and kidney were stained with hematoxylin and eosin; sections of kidney were also stained with Masson and periodic-acid-Schiff (PAS) stains.

At time of sacrifice, all experimental animals appeared ill, with ruffled fur and relative immobility. The chest cavities uniformly contained blood-tinged fluid, and two or three hemorrhagic infarcts of each lung were noted. One animal had an infarct of the anterior wall of the myocardium. The kidneys appeared pale and bloodless; spleen and liver seemed uninvolved.

Renal lesions were present in all experimental animals; glomeruli were swollen and presented a striking occlusion of the capillary bed with an eosinophilic, hyaline, fibrin-like material (Fig. 1). Little inflammatory reaction was apparent, although occasional polymorphonuclear leukocytes and small areas of necrosis were noted. The tubules contained proteinaceous material but were otherwise unremarkable. Occasional small

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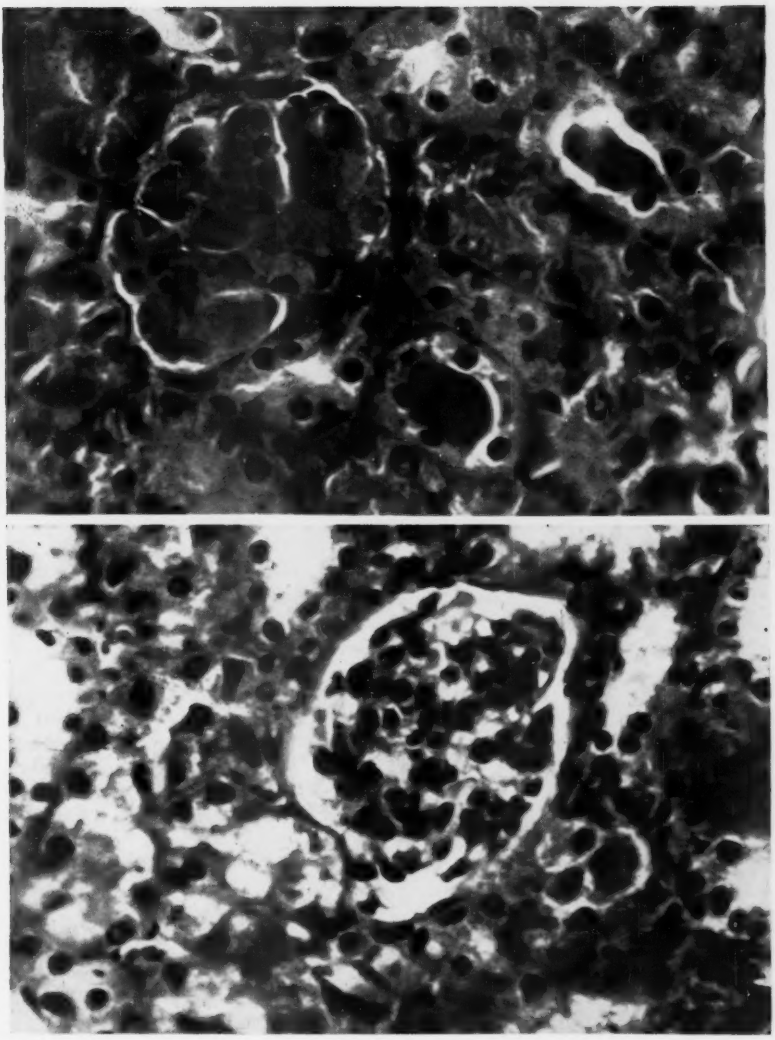

FIGS. 1 & 2.

FIG. 1. Mouse kidney 24 hours after three injections of type 1 M protein. Note the marked occlusion of the glomerular capillary loops with an eosinophilic, amorphous, fibrin-like material. Protein is also seen in tubular lumina. x450.

FIG. 2. Mouse kidney 24 hours after three injections of human serum albumin. x450.

eosinophilic masses of fibrin-like material were present in the spleen. Microscopically the liver was normal. Organs of control mice, injected with HSA, were grossly and microscopically normal (Fig. 2).

Scarcity of purified M protein precluded a larger series of animals at high dosage, but 10 additional mice have received from 3 to 15 mg. of type 1 M protein in single and multiple intravenous injections with consistent demonstration of renal lesions. In animals receiving smaller amounts, pathology was limited to minimal glomerular lesions with no other organ involvement. Capillary thrombi had the characteristic staining reaction of fibrin.

## DISCUSSION

Intravenous administration of a partially purified preparation of streptococcal M protein uniformly resulted in glomerular lesions. The nature of the capillary thrombi suggests that these lesions resulted from localization of intravascularly formed complexes of M protein and fibrinogen within the glomerular capillary bed. Experimental data support this view: (i) partially purified preparations of M protein precipitated fibrinogen *in vitro*[2]; (ii) staining reactions of the capillary thrombi were similar to those of fibrin; (iii) intravenously injected M protein has been shown to localize in the glomerular tufts.[4]

Human glomerulonephritis is well known to be associated with preceding streptococcal infection. Although acute renal lesions produced by M protein are not similar histologically to those seen in human glomerulonephritis, localization in the glomerular capillary bed of streptococcal M protein may be a factor initiating the nephritic process in the sensitive host.

## SUMMARY

Renal glomerular lesions resulted from the intravenous injection of partially purified streptococcal M protein. The lesions are capillary thrombi of an amorphous, eosinophilic, fibrin-like material. It is suggested that intravascular precipitation of M protein and fibrinogen with subsequent renal localization is involved in the pathogenesis of these lesions.

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AQUATIC PHYCOMYCETES. 2d ed., and enlarged. By Frederick K. Sparrow, Jr. Ann Arbor, University of Michigan Press, 1960. 1,187 pp. $22.50.

This volume is a revised, greatly expanded, and thoroughly admirable successor to a first edition (1943) which has been of inestimable value in facilitating the labors and stimulating research among those concerned with various aspects of aquatic microbiology. The present authoritative work is a welcome and imposing addition to biological literature by an investigator internationally renowned for his contributions to our knowledge of aquatic fungi.

The logical design of the earlier edition has been retained but much new and valuable information has been poured into the old format. In addition to the inclusion of freshwater and marine Phycomycetes reported since the publication of the first edition, the scope of the treatise has been broadened significantly. Thus, the present volume describes species of *Pythium* (in key form) and *phytophthora* known to exist as water molds in natural habitats, genera of the Saprolegniaceae and citation of new taxa described since the publication of Coker (1923) and Coker and Mathews (1937), parasites of desmids belonging to the genus *Ancylistes* of the Entomophthorales, as well as the names and citations of species of *Physoderma* which parasitize marsh and aquatic vascular plants. Full descriptions are also given of those members of the Plasmodrophorales which attack aquatic cryptogams.

A comprehensive introduction provides a lucid account of various biological aspects of aquatic Phycomycetes. Falling within the purview of this section are discussions of their classification and phylogeny, zoospore types and their taxonomic importance, hydrobiological relationships, geographic distribution, and procedures relating to their isolation, cultivation, and preservation. The section concludes with a key to the ten orders of aquatic Phycomycetes (Uniflagellate series—Chytridiales, Blastocladiales, Monoblepharidales, Hyphochytriales; Biflagellate series—Plasmodiophorales, Saprolegniales, Leptomitales, Lagenidiales, Peronosporales; Conidial series —Entomophthorales). These orders are then considered in individual sections which together constitute the major portion of this compendium.

The systematic treatment of each order is preceded by a consideration of such matters as the taxonomic history of the group, descriptions of reproduction, morphology, and development, cytogenetic data, parasitic relationships, and references to relevant physiological studies. It is to Dr. Sparrow's credit and no mean stylistic feat that these expository sections are crisp and eminently readable in spite of the copious documentation. Indeed, these accounts make it clear that the massiveness of the new edition (double the size of the older one) results from the information explosion rather than from inattention to the principles of "literary girth control." The methodical taxonomic treatment of the order follows the more general discussion and numerous line illustrations and keys to families, and species are provided. Together with technical diagnoses of individual species further data are supplied relating to substrata, collectors, bibliographical citation of collections, countries from which the organisms have been reported, and notable features of intrinsic biological interest. Taxa published between the date

limit of this book (1 January 1955) and the end of 1957 are included in special sections interspersed at relevant points in the text, and the results of more recent investigations are noted in footnotes.

Following all of the systematic accounts an index of substrata is provided listing plants, animals, and organic substances together with the phycomyceteous fungi associated with them. A bibliography with more than 1,200 listings and a general index conclude this work.

Dr. Sparrow's volume is a major achievement which will long benefit those concerned with aquatic biology.

SOLOMON GOLDSTEIN

PSYCHOANALYSIS AND MORAL VALUES. By Heinz Hartmann. New York, International Universities Press, Inc., 1960, 109 pp. $3.00.

This is an expanded version of a lecture which was part of the Freud Anniversary Series in 1959. Its simply descriptive title should not lead to any conclusion that this is a "popularized" treatment of an immense area. Those previously familiar with Dr. Hartmann's contributions to psychoanalysis know that very little which flows from his pen is banal. This work is wholly concordant with his persistent attempts to refine the conceptions and boundaries of psychoanalytic theory. His past successful endeavors in this regard have won him a high place in the roster of psychoanalytic theorists and solid esteem for the fertility of his mind. In the opening sentence he warns that he is about to cover a variety of partly heterogeneous subjects, which is indeed the case. But the profundity of his grasp of the topics results in a synthesis which is impressive, informative, and stimulating. Hartmann has never been easy to read, but it is pleasant to report that this essay is considerably more lucid than some of his earlier publications. This is a particularly fortunate attribute here because undoubtedly it will have—and should have—a wider circulation than his metapsychological writings.

During the first part, and repeatedly from different directions throughout, the point is established that psychoanalysis cannot and does not provide a philosophy of life nor, any more than any scientific enterprise, can it decide which values should be supreme. This remains true despite the efforts of some to "evangelize" or "substantiate" a moral system with psychoanalytic data. In the positive sense he painstakingly elaborates what can be and is the contribution of psychoanalysis to problems of morals. Briefly stated, he proposes that psychoanalysis can provide a systematic psychology of moral aims and imperatives: "We can judge their psychological implications. We can form a scientific opinion on what strivings they will or will not gratify, on their synergisms and antagonisms with other mental tendencies."

Of the remainder of the "heterogeneous subjects" treated, it must suffice merely to mention "health ethics," the ontogenesis of moral systems, and the effects of treatment on moral systems. Of great importance are several suggestions for psychoanalytic, and possibly interdisciplinary, research.

JOHN W. HIGGINS

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